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Limnology of Canyon Ferry Reservoir

II. Phytoplankton Standing Crop and Primary Production

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ABSTRACT

In general, one μg chlorophyll was equivalent to 0.5 mm^3 cell volume and 0.12 mg ash-free dry weight. The average seston content consisted of 34.5% phytoplankton, 9.8% zooplankton, and 55.7% detritus. Optimal photosynthesis per unit chlorophyll and cell volume averaged $0.39\text{ }\mu\text{ mole O}_2/\mu\text{g chl/hr}$ and $0.86\text{ }\mu\text{ mole O}_2/\text{mm}^3/\text{hr}$, respectively. The average euphotic zone photosynthetic rate was 52% of the optimal rate. Chlorophyll content per unit volume of cells and photosynthesis per unit chlorophyll or cell volume decreased with increase in population size. Evidence was found of an interacting effect of temperature, light intensity, and phosphates on photosynthesis. An empirical method was described for estimating photosynthesis on the basis of chlorophyll content, optimal rate of photosynthesis per unit chlorophyll, extinction coefficient, and the ratio of euphotic zone photosynthesis to optimal photosynthesis. Determinations of phytoplankton respiration were made, and net euphotic zone photosynthesis was computed for various sizes of chlorophyll standing crop. It was concluded that the most frequently occurring values of chlorophyll standing crop were most likely to produce a maximum net production and to be at a steady state level.

INTRODUCTION

The attempt to measure primary production in relation to phytoplankton standing crop has involved three measures: (1) cell volume, (2) seston, and (3) chlorophyll (Verduin 1956b). Data on rate of photosynthesis based on cell volume and ash-free dry weight have been presented by Verduin (1952, 1954, 1956a, and 1956b) and McQuate (1956). Information on chlorophyll: photosynthesis ratios has been provided by Edmondson (1955), Manning and Juday (1941), Riley (1941), and Ryther and Yentsch (1957). Photosynthesis as related to seston has been discussed by Jackson and McFadden (1954). To my knowledge, only Riley (1941) has presented data in which all three measures of standing crop are related to primary production.

One of the objectives of the present

study was to obtain information on the relationship of these three quantities to primary production. The information presented was collected during the periods April to October, 1957, and April to September, 1958.

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METHODS

Samples were collected and handled in a manner similar to that described in a previous paper (Wright 1958), except that clear bottles were suspended at two and one-half

foot intervals from the surface to 20 feet, and one bottle was placed at the 25-foot depth.

Volume of phytoplankton was determined by counting and measuring the algal cells with a Zeiss-Winkel inverted microscope. The plankton which was fixed with Lugol's solution was allowed to sediment overnight in a five ml cylindrical chamber. Counts were made at 20, 80, 320, and 800 $\times$ depending upon the size of the alga. Dimensions of the cells or colonies were estimated with a Whipple micrometer disk, and volumes were computed using appropriate formula. When large cells or colonies were enumerated at 20 $\times$ magnification, the entire surface of the counting chamber was scanned. At higher magnifications, either 50 or 100 fields were counted, depending upon the density of the species present.

Samples for chlorophyll determination were obtained by filtering 500 ml of the sample through a type AA 047 mm "millipore" filter. The filter was treated as described by Creitz and Richards (1955). Upon return to the laboratory, the filter was dissolved in five ml of 90% acetone and held in the refrigerator overnight. The following morning, the acetone extract was separated from the residue by centrifugation in a clinical centrifuge. Chlorophyll concentrations were determined with a Klett-Summerson colorimeter using a red filter. In 1958, the method of chlorophyll determination using a Beckman DU spectrophotometer as described by Richards with Thompson (1952) was employed. It was found that 0.28 μg chlorophyll *a*/L was equivalent to one Klett unit. A comparison was made between chlorophyll content of "millipore" filter samples and centrifuge samples on several occasions.

Samples of seston were obtained by twice centrifuging one liter of water through a Foerst continuous-flow centrifuge at a rate of one liter per six minutes. The samples obtained during 1958 were dried for 24 hours or longer at 100°C in "Vycor" crucibles, weighed, and then ashed at 600°C for 30 minutes and weighed again.

A series of net plankton samples was obtained by towing a #20 plankton net. The concentrate was mixed with lake water

from which a 250-ml glass-stoppered black bottle was filled and suspended in the lake for six hours. The oxygen decline in the black bottle was used as a measure of respiration. A 125-ml aliquot of the sample was filtered through a "millipore" filter for chlorophyll determination, and another 125-ml aliquot was preserved with formaldehyde and later filtered through a filter crucible with a maximum pore size of three microns. Dry weight and ash-free dry weight were determined from this sample. A third aliquot was fixed with Lugol's solution for zooplankton counts.

Zooplankton samples were collected by oblique tows with a Clarke-Bumpus plankton sampler from 25 feet to surface using a #10 net. The formalin fixed samples were made up to 500 ml, and all animals in one ml aliquots were counted.

Phosphate was determined on "millipore" filtered samples, using the ammonium molybdate-stannous chloride method. The intensity of color formation was determined colorimetrically, using a 4-cm cell.

Temperatures were measured with a Tri-R electronic thermometer in 1957 and with a bathythermograph in 1958. Light penetration data were obtained with a G. M. Manufacturing Company, model 15M-02 submarine photometer which employs a Weston photronic cell. During each photosynthesis run, surface light intensity was estimated by averaging readings taken at one-half hour intervals with the photometer. Average light intensity at each clear bottle depth was computed on the basis of the extinction coefficient and average surface light intensity.

RELATIONSHIP BETWEEN CHLOROPHYLL, CELL VOLUME, AND ASH-FREE DRY WEIGHT OF PHYTOPLANKTON

The histogram (Fig. 1) shows the frequency at which various chlorophyll/cell volume ratios occurred. Approximately 60% of the samples was included in the range between 1-3 μg chlorophyll/mm³ cells. The median ratio was 2 μg chl/mm³ cells.

The regression equation between chlorophyll and cell volume, $C = 1.68V + 2.05$, indicates that there is a non-linear relation-

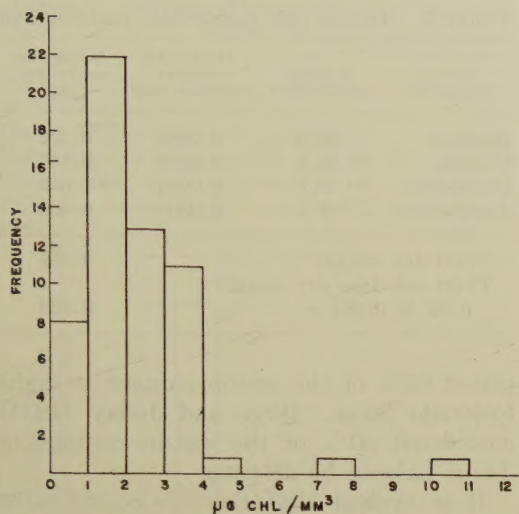


FIG. 1. Frequencies of the values of chlorophyll/cell volume ratios.

TABLE 1. Size of standing crop compared with chlorophyll content and rate of optimal photosynthesis per unit cell volume

Standing crop mm³/l		Average Chlorophyll/cell volume ratio µg chl/mm³	Average rate of optimal photosynthesis µmole O ₂ /mm³/hr
Range	Average		
0-2.5	1.75	3.53	1.48
2.6-5.0	3.72	2.09	0.81
5.1-7.5	6.18	1.80	0.55
7.6-10.0	8.38	1.76	0.46
>10.1	16.10	1.42	0.48

ship between chlorophyll and cell volume, since chlorophyll would be 2.05 µg/L when cell volume was zero. Examination of Table 1 shows that, as the size of the standing crop in terms of cell volume increased, the ratio of chlorophyll to cell volume decreased. This relationship suggests that chlorophyll synthesis becomes limited by some factor (perhaps nitrogen) as the population increases.

The frequency distribution of the ratios of ash-free dry weight to chlorophyll is shown in Figure 2. Of the 44 samples, 73% ranged between 0.02 and 0.20 mg ash-free dry weight/µg chl. The median ratio of 0.12 is very close to the average ratio of 0.11 for marine plankton computed from the data of Harris and Riley (1956).

Figure 3 shows the frequency at which various ratios of ash-free dry weight to cell

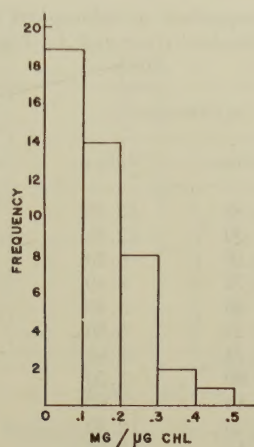


FIG. 2. Frequencies of the values of ash-free dry weight/chlorophyll ratios.

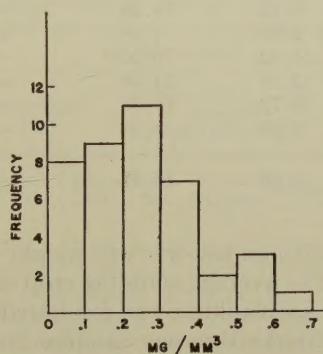


FIG. 3. Frequencies of the values of ash-free dry weight/cell volume ratios.

volume occurred. The most frequently occurring ratios were in the range between 0.2 and 0.3 mg ash-free dry weight/mm³.

On the basis of the data presented, the best estimate of the average relationship between the three indices of phytoplankton standing crop would be 1.0 µg chl = 0.5 mm³ cells = 0.12 mg ash-free dry weight.

Millipore filters were considerably more efficient in retaining chlorophyll than the centrifuge method (Table 2). The average of 20 millipore samples was 13.5 compared to 9.7 µg/L for the centrifuge samples.

COMPOSITION OF THE SESTON

The average ash-free dry weight of seston samples collected during 1958 was 2.35 mg/L. The average chlorophyll content of these samples was 6.8 µg/L, which would be

TABLE 2. Comparison of chlorophyll retention by Foerst plankton centrifuge and AA type millipore filters

μg Chlorophyll/L		Per cent difference	
Centrifuge	Millipore		
12.88	12.88	-00	
7.00	12.88	-46	
6.16	8.40	-27	
6.72	8.40	-20	
7.56	8.40	-11	
2.24	5.60	-60	
5.74	7.84	-27	
5.60	7.70	-27	
6.16	7.67	-20	
6.25	12.50	-50	
7.56	7.67	- 3	
6.16	10.64	-42	
16.80	21.84	-23	
8.68	11.20	-23	
8.12	11.20	-28	
2.80	1.68	+40	
54.32	70.56	-23	
12.88	24.08	-47	
6.72	10.08	-33	
3.36	8.40	-60	
Mean	9.69	13.48	-27

equivalent to an ash-free dry weight of 0.81 mg/L. The average standing crop of larger species of zooplankton was computed on the basis of Clarke-Bumpus samples from the euphotic zone. On the basis of the calculations presented in Table 3, the average ash-free dry weight of zooplankton was 0.23 mg/L. This figure does not include the smaller zooplankton forms, but their weights would be negligible and would not alter the order of magnitude. On the basis of these figures, 34.5% of the seston was phytoplankton, 9.8% was zooplankton and the remainder (55.7%) could be considered as detritus.

Davis (1958) presented data on the volume of phytoplankton, zooplankton, and detritus for western Lake Erie. On the basis of his figures, phytoplankton comprised 50.9%, zooplankton 28.0%, and detritus 21.1% by volume of the suspended organic material of the Lake Erie samples. Riley (1940) concluded that phytoplankton in Linsley Pond averaged 18.5% of the seston, zooplankton 2.0, and detritus 79.5. Pennak (1955) estimated that detritus com-

TABLE 3. Average 1958 zooplankton standing crop

Organism	Average number/liter	Average dry weight/individual (mg)	Average dry weight (mg)/liter
<i>Daphnia</i>	20.0	0.0086	0.172
<i>Cyclops</i>	46.5	0.0040	0.186
<i>Diaptomus</i>	2.1	0.0086	0.018
<i>Asplanchna</i>	6.7	0.0008	0.005
Total dry weight			0.381
Total ash free dry weight:			
$0.60 \times 0.381 =$			0.229

prised 65% of the seston content in eight Colorado lakes. Birge and Juday (1934) considered 50% of the seston content of Trout Lake to be detritus.

It is evident that there is considerable variation among the estimates of detritus content of the few lakes in which an attempt has been made to estimate its magnitude. On the basis of these scanty data, there seems to be a fair degree of correlation between the size of the lake and the detritus/seston ratio. In Table 4 the areas of the lakes mentioned are compared with the detritus/seston ratio. It will be noted that the greater the area of the lake, the smaller the ratio of detritus to seston. This is of importance in view of the divergence of opinion expressed by Pennak (1955) and Davis (1958) concerning the importance of detritus in secondary production.

As Edmondson (1957) pointed out, detritus may have three principal sources, (1) terrestrial, (2) littoral, (3) planktonic. If the detritus is of planktonic origin, then discussion as to the relative importance of detritus vs. plankton is absurd. If the source is littoral, the problem of food consumption by the zooplankton is still a problem of primary production within the lake. However, if the major source of detritus is terrestrial, the energy economy of the zooplankton could proceed at a faster rate than could be supported by phytoplankton production. It would be difficult to separate littoral- and terrestrial-derived detritus from each other; however, the importance of both would diminish with increase in volume of the lake, as the volume would tend to increase as the square of the circumference. Therefore one would expect the ratio of

TABLE 4. *Detritus/stonon ratios for lakes of various size*

Lake	Area in hectares	Detritus/stonon ratio
Western Lake Erie	3.3×10^6	0.21
Canyon Ferry Reservoir	1.4×10^4	0.56
Trout Lake	6.5×10^2	0.50
Colorado Lakes	1.5-205.2	0.65
Linsley Pond	9.4	0.80

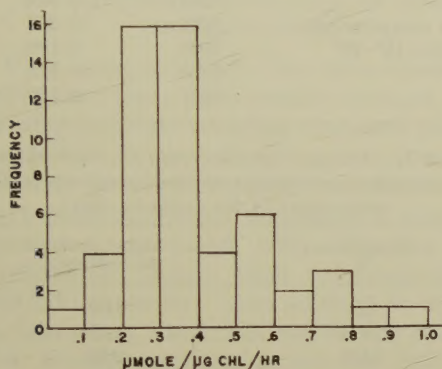


FIG. 4. Frequencies of the values of optimal photosynthesis/chlorophyll ratios.

detritus to stonon to vary with lake size as indicated in Table 4. It would appear that the disagreement in conclusions reached by Pennak and Davis is more apparent than real, since they were working with lakes of different sizes.

OPTIMAL RATE OF PHOTOSYNTHESIS

Optimal photosynthesis averaged $0.39 \mu\text{mole O}_2/\mu\text{g chl/hr}$ and ranged from 0.10 to 0.91. The average value is equivalent to the value of 3.7 g C/g chl/hr (Ryther and Yentsch 1957), assuming a photosynthetic quotient of 1.25. The frequency distribution of chlorophyll-based ratios is shown in Figure 4. Over 59% was in the range between 0.20 and $0.40 \mu\text{mole O}_2/\mu\text{g chl/hr}$. The correlation coefficient ($r = 0.910$) between chlorophyll and optimal photosynthesis was highly significant. On the basis of the chlorophyll/ash-free dry weight ratio, optimal photosynthesis would be $3.25 \mu\text{moles O}_2/\text{mg ash-free dry weight/hr}$.

Volume-based rates averaged $0.86 \mu\text{moles O}_2/\text{mm}^3/\text{hr}$ and ranged between 0.20 to 3.86. The frequency distribution of ratios based on cell volume is shown in Figure 5.

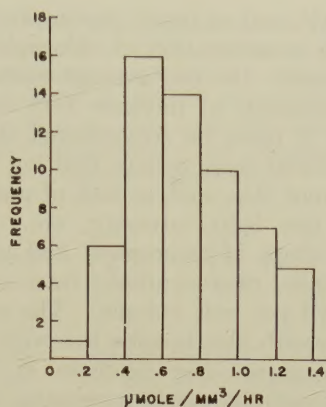


FIG. 5. Frequencies of the values of optimal photosynthesis/cell volume ratios.

TABLE 5. *Chlorophyll standing crop compared with average rates of optimal and euphotic zone photosynthesis and average daily rate of change of chlorophyll*

Number of samples	Chlorophyll $\mu\text{g/l}$		Photosynthesis per unit chlorophyll $\mu\text{mole O}_2/\mu\text{g chl/hr}$			Rate of change of chlorophyll standing crop $\mu\text{g chl}/\mu\text{g chl/day}$
	Range	Average	Optimal	Euphotic zone	Ratio	
4	0-2.5	1.68	0.70	0.45	0.64	+0.230
10	2.6-5.0	4.37	0.48	0.23	0.48	+0.024
20	5.1-10.0	7.51	0.38	0.18	0.47	-0.006
13	10.1-15.0	11.65	0.34	0.15	0.44	-0.035
6	15.1-25.0	18.03	0.25	0.11	0.44	-0.067

The correlation coefficient ($r = 0.913$) between cell volume and optimal photosynthesis is highly significant.

If a comparison is made between cell volume per liter and optimal photosynthesis per unit cell volume, it is apparent that, paralleling the decrease in chlorophyll per unit cell volume with increasing cell volume, there is also a decrease in the photosynthetic rate per unit volume (Table 1).

In Table 5, standing crops of chlorophyll are compared with the corresponding chlorophyll-based rates of optimal and euphotic zone photosynthesis and average daily rate of change of chlorophyll. Although there was considerable scatter in the data on which these averages were based, nevertheless there was a consistent trend in their relationship. Low standing crops of chlorophyll generally had high rates of photosynthesis per unit

chlorophyll, and as noted previously (Table 1) a high concentration of chlorophyll per unit volume. On the average such crops had a tendency to increase very rapidly, although it must be remembered that for any particular date various limiting factors may prevent this, such as lack of nutrients, grazing, low light intensity, etc. Larger standing crops of chlorophyll had progressively smaller photosynthetic rates and less chlorophyll per unit volume. The average rate of growth also became less with larger standing crops. The decrease in photosynthetic activity with increasing size of standing crop has also been observed in Sandusky Bay (McQuate 1956) and in Lake Erken (Rohde *et al.* 1958).

The data in Table 5 seem to indicate that when one randomly samples phytoplankton populations at intervals over an extended period of time, some of the populations sampled are young and actively growing, others are in a steady-state condition, and others senescent. Populations ranging between 5 and 10 $\mu\text{g chl/L}$ seem to be at an approximate steady-state level corresponding to an average set of conditions occurring during the time these populations were selected. It is interesting to note (Table 5) that the most frequently occurring populations had chlorophyll concentrations ranging between 5.1 and 10.0 $\mu\text{g/L}$. Sixty-four per cent of the populations had less than 10 $\mu\text{g chl/L}$, which is within the range of standing crops having photosynthetic rates that generally resulted in an increase of standing crop or the maintenance of a steady state.

EUPHOTIC ZONE PHOTOSYNTHESIS

The average euphotic zone photosynthetic rate was 52% of the optimal rate, or 0.20 $\mu\text{moles O}_2/\mu\text{g chl/hr}$. The correlation coefficient between optimal photosynthesis and the corresponding euphotic zone rate was high ($r = 0.850$). Size of standing crop affected the average rate of euphotic zone photosynthesis as well as the optimal rate (Table 5). The greater the standing crop the less was the average rate of photosynthesis per unit chlorophyll in the euphotic zone. With increase in chlorophyll standing crop, the ratio of euphotic zone photosyn-

TABLE 6. *Effect of light intensity on the average rate of euphotic zone photosynthesis per unit chlorophyll with respect to temperature*

Temperature °C	Average surface light intensity (microamperes)	Average euphotic zone photosynthesis $\mu\text{mole O}_2/\mu\text{g}$ chl/hr
Low temperature series 0-14°	8606	0.092
	5932	0.129
	4556	0.231
	3234	0.324
High temperature series 16°-23°	9209	0.263
	8003	0.199
	6603	0.138
	4651	0.139

TABLE 7. *Average light intensity at which optimal photosynthesis occurred compared to average water temperature of the euphotic zone*

Temperature °C	Light intensity microamperes
4	1180
9	1424
14.5	2093
18.5	3510
21.3	3634

thesis to optimal photosynthesis declined also.

An interesting interaction between surface light intensity, temperature, and euphotic zone photosynthesis was found. The populations were separated into two groups on the basis of temperature—a low temperature group (4-14°C) and a high temperature group (16-23°C). The effect of increasing light intensity on euphotic zone photosynthesis in the low temperature series was just the reverse of that in the high temperature series (Table 6). High light intensities markedly inhibited the rate of photosynthesis of cold temperature populations and increased the rate of photosynthesis in warm temperature populations.

The interaction between light and temperature is illustrated further when a comparison is made between temperature and the average light intensity at which optimal photosynthesis occurred (Table 7). As the temperature increased, optimal photosynthesis occurred at higher light intensities.

Rohde *et al.* (1958) observed a similar relationship in Lake Erken, and concluded that "the phytoplankton is able to com-

pensate to a very large extent, seasonal changes in light conditions by means of its successive adaptation. Owing to this ecological as well as other physiological reasons, the concept of a constant light optimum has to be replaced by a concept of the movable optimum."

RELATIONSHIP BETWEEN AVERAGE EUPHOTIC ZONE PHOTOSYNTHESIS AND OPTIMAL PHOTOSYNTHESIS

It is apparent from the data discussed above that there is no simple constant relationship between standing crop and photosynthesis. Instead there is a complex relationship between variable populations of phytoplankton which undergoes a seasonal succession correlated with changing environmental factors such as temperature and light intensity. Any attempt to derive an expression which will enable one to predict primary production on the basis of standing crop must involve the collection of a large number of samples covering a wide range of standing crops and environmental conditions. From an empirical consideration of such data it should be possible to derive expressions of relationship that will at least enable one to predict an average rate of production on a monthly or perhaps weekly basis. On the basis of the limited data available, an attempt has been made to predict average monthly euphotic zone photosynthesis on the basis of the following equation:

$$\sum P = \frac{4.6}{k} \times F \times P_{\text{opt}} \quad (1)$$

in which $\sum P$ is euphotic zone photosynthesis, k is the extinction coefficient, P_{opt} is optimal photosynthesis, and F is the ratio of euphotic zone photosynthesis to optimal photosynthesis on a unit volume basis.

The expression $\frac{4.6}{k}$ defines the depth of the euphotic zone as the depth of a layer of water lying above the plane at which light intensity is 1% of surface light intensity. If P_{opt} is expressed as mmole O_2/m^3 , then P would be in terms of mmole O_2/m^3 .

Numerical values of F were computed from observed values of optimal and eu-

TABLE 8. Average monthly values for euphotic zone photosynthesis ($\sum P$), optimal photosynthesis (P_{opt}), extinction coefficient (k), and the ratio of euphotic zone to optimal photosynthesis (F)

These values are compared with average light intensity, temperature, and phosphate phosphorus concentration.

Month	$\sum P$ mmole $\text{O}_2/\text{m}^3/\text{hr}$	k	P_{opt} mmole $\text{O}_2/\text{m}^3/\text{hr}$	F	Light micro- amperes	Temperature $^{\circ}\text{C}$	Phosphorus mmole P/m^3
April	10.18	0.64	4.04	0.34	4873	4.8	1.06
May	7.82	0.64	2.27	0.44	5985	10.7	0.54
June	11.27	0.66	2.75	0.49	7804	16.8	0.48
July	10.27	0.74	2.92	0.58	6704	18.9	0.59
Aug.	10.81	0.67	2.69	0.59	7902	20.9	0.12
Sept.	12.80	0.79	3.68	0.60	6315	18.6	0.70
Oct.	5.91	0.71	1.44	0.59	3839	13.8	0.61
Mean	9.87	0.69	2.83	0.52			

photoc zone photosynthesis and the extinction coefficient for each sampling date. All the values for each month were then averaged (Table 8).

It will be noted that there is no clearly defined relationship between light intensity and F when these two variables are considered alone. The increase in F seems to be better correlated with temperature from April to July. However, the F value for August should be higher if the temperature trend was extrapolated. The extremely low phosphate concentrations during August may have been responsible for this discrepancy. Furthermore, the higher phosphate concentrations in September and October may possibly have counteracted the effect of declining radiation and temperature, resulting in higher values of F than could be expected on the basis of the trend from April to July. Admittedly the data are inadequate to substantiate this hypothesis, but certainly some relationship is indicated between temperature, light, nutrients, and photosynthesis that warrants further study.

On the basis of 24 hr C^{14} measurements, Rohde *et al.* (1958) found the following simple relationship between daily euphotic zone photosynthesis and optimal photosynthesis:

$$\sum a = (2.4 \text{ to } 2.7) \frac{a_{\text{opt}}}{\epsilon} \quad (2)$$

TABLE 9. A comparison between monthly values of total relative photosynthesis as computed by the method described, using a 12-hour photoperiod and Ryther's (1956) method

Month	Total relative photosynthesis	
	4.6 <i>FT</i>	<i>R_s</i> (Ryther)
April	18.8	21.0
May	24.3	25.0
June	27.0	26.0
July	32.0	25.0
Aug.	32.5	24.0
Sept.	33.1	22.0
Oct.	32.6	20.0
Mean	28.6	23.3

The constant (2.4–2.7) is equivalent to 4.6 *F* in Equation (1). The average value of 4.6 *F* would be 2.4, which agrees very nicely with the values reported by Rohde and his co-workers. However the range of values between 1.6 for April and 2.8 for August is greater than the range of 2.4–2.7 which they found.

If one assumed that the factor *F* applied to the entire photosynthetic period as well as to the six-hour photosynthetic periods used in this study, daily euphotic zone photosynthesis could be calculated from the following equation, where *T* is the number of hours of daily photosynthesis:

$$\sum P = \frac{4.6}{k} \times F \times T \times P_{\text{opt}} \quad (3)$$

The expression 4.6 *FT* is equivalent to total relative euphotic zone photosynthesis (*R_s*) as used by Ryther (1956).

Since Doty and Oguri (1957), Verduin (1957), and Yentsch and Ryther (1957) have found variations in the hourly rate of photosynthesis during the day, one cannot simply substitute the number of hours of daylight for *T* with any degree of confidence. Furthermore, as pointed out previously in the discussion concerning the effect of light and temperature on photosynthesis, there is no fixed light optimum for photosynthesis. In view of this uncertainty it seems reasonable to assume a 12-hour photoperiod for the months of April to October as Verduin (1956b) has suggested. Values of 4.6 *FT*

computed on a 12-hour basis are shown in Table 9 and are compared with values of *R_s* according to Ryther's (1956) method. These latter values were computed on the basis of radiation measurements from the Twin Falls, Idaho, weather station (Duggar 1936). The two estimates agreed closely for April, May, and June, during the period when diatoms were dominant in the phytoplankton; but Ryther's method gave lower values for July, August, September, and October. One reason for the discrepancy may be that Ryther's treatment overestimates surface inhibition by high light intensities during this period when blue-green algae dominate the plankton. If the values for total relative photosynthesis (*R_s*) were adjusted to correspond to the relationship between photosynthesis in dinoflagellates and light intensity (Ryther 1956), they would more nearly correspond to the values found in this study.

CALCULATION OF EUPHOTIC ZONE PHOTOSYNTHESIS ON THE BASIS OF CHLOROPHYLL CONTENT

Euphotic zone photosynthesis has been calculated from the relationship

$$\sum P = \frac{4.6}{k} \times F \times T \times C \times R_{\text{opt}} \quad (4)$$

where *C* is chlorophyll concentration (mg/m³), and *R_{opt}* is the optimal rate of photosynthesis per unit chlorophyll (mmole O₂/mg chl/hr).

Euphotic zone photosynthesis was calculated two ways. The average rate of optimal photosynthesis (0.39 mmole/mg chl/hr) was used in one set of calculations. Variable rates of optimal photosynthesis dependent upon size of standing crop (Table 5) were used in another set. The results expressed as average values per month are compared with average measured values in Table 10.

The average monthly deviation from actual measurements as calculated by using a constant rate of optimal photosynthesis was 23%. When variable rates were used, the average deviation of calculated values from measured values was 13%. It appears that better estimates of euphotic zone photosynthesis may be secured by correcting for

the effect of size of standing crop on the rate of photosynthesis.

In Table 11 are given daily photosynthesis values from *in situ* light and dark bottle oxygen experiments (Ryther and Yentsch 1957) and from *in situ* changes in dissolved oxygen in the water column or a combination of the two methods (Riley 1956, Conover 1956, and Riley 1957). The data of Ryther and Yentsch, which were given in terms of g C/m²/day, were converted to mmoles O₂/day by assuming a photosynthetic quotient of 1.25, which was used by them to convert oxygen production to carbon production. These values are compared

with values of daily photosynthesis calculated from Equation (4), assuming a 12-hour photoperiod. In the case of Riley's (1957) data, the average rate of optimal photosynthesis per unit volume (0.86 mmole/ml/hr) was used instead of chlorophyll-based rates. On the whole, there is close agreement between photosynthesis values calculated by the method outlined above and the measured values. Agreement is best where a large number of values is averaged as in the case of the Long Island Sound and Sargasso Sea data.

PHYTOPLANKTON RESPIRATION AND NET PRODUCTION

The determination of phytoplankton respiration under natural conditions is most difficult since any measurement of respiration of the phytoplankton community will include respiration of the zooplankton and bacteria as well. An approximate estimate of phytoplankton respiration was obtained by measuring the oxygen uptake of net concentrates placed in black bottles and suspended in the lake for six hours.

The average rate of respiration of the samples of low zooplankton content was 0.033 μ moles O₂/μg chl/hr and ranged from 0.004 to 0.118. Most of the samples (62%) were between 0.02 and 0.04 μ moles/μg chl/hr (Figure 6).

In Table 12, gross photosynthesis, respiration, and net photosynthesis are compared for several ranges of euphotic zone standing

TABLE 10. A comparison of measured values of euphotic zone photosynthesis with values estimated on the basis of chlorophyll concentration, optimal rate of photosynthesis per unit chlorophyll, and the extinction coefficient

Month	Number of samples	Average chlorophyll mg/m ³	Averages by month of hourly euphotic zone photosynthesis (mmole O ₂ /m ² /hr)		
			Average rate of optimal photosynthesis used	Variable rate of optimal photosynthesis used	Actual measured rates
April	5	10.0	8.8	8.1	10.2
May	7	8.7	10.8	9.8	7.8
June	9	9.4	12.5	11.3	11.3
July	12	16.7	15.5	12.4	10.9
Aug.	10	6.1	9.6	10.4	10.8
Sept.	7	9.3	12.7	11.4	12.8
Oct.	4	2.1	4.0	6.2	7.6
Mean			10.6	9.9	10.2

TABLE 11. A comparison of measured rates of photosynthesis from various sources and values computed from equation 4

Place	Reference	K	Chlorophyll mg/m ³	Cell volume ml/m ³	Photosynthesis mmole O ₂ /m ² /day	
					computed	measured
Long Island Sound						
1952-53	Riley (1956)	0.63	6.20		110	113
1953-54	Conover (1956)	0.68	6.74		110	106
Sargasso Sea	Riley (1957)	0.11		0.132	30	23
Friday Harbor, Wash.	Ryther & Yentsch (1957)	0.13	1.0		86	35
East Sound, Wash.	Ryther & Yentsch (1957)	0.30	15.0		589	521
Gulf of Alaska	Ryther & Yentsch (1957)	0.10	2.5		280	156
Woods Hole Harbor	Ryther & Yentsch (1957)	0.27	3.35		139	169
Woods Hole Harbor	Ryther & Yentsch (1957)	0.27	1.69		70	82
Woods Hole Harbor	Ryther & Yentsch (1957)	0.24	2.80		131	114
Average					168	147

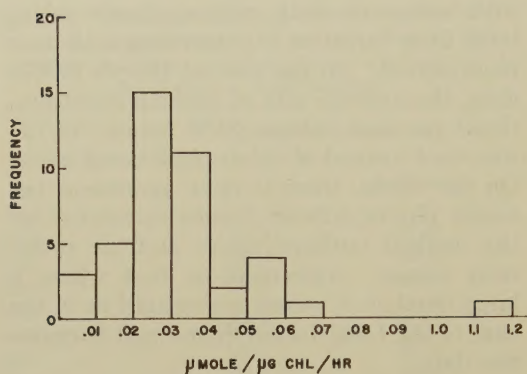


FIG. 6. Frequencies of the values of respiration/chlorophyll ratios.

TABLE 12. A comparison of euphotic zone chlorophyll standing crop, gross photosynthesis, respiration, and net photosynthesis

Chlorophyll mg/m ³		Gross photo- synthesis mmole O ₂ /m ² /hr	Respiration mmole O ₂ /m ² /2 hr	Net photo- synthesis mmole O ₂ /m ² /hr
Range	Average			
0-2.5	1.7	4.6	1.0	3.6
2.6-5.0	4.2	7.9	2.3	5.6
5.1-10.0	7.9	10.7	4.3	6.4
10.1-15.0	12.2	12.4	6.7	5.7
15.1-20.0	15.7	14.4	8.6	5.8
20.1-25.0	23.0	16.9	12.6	4.3
70.6	70.6	11.7	38.7	-27.0

crop. The values for gross photosynthesis are averages of clear bottle measurements of photosynthesis for each range of standing crop. The respiration figures were computed by averaging the products of standing crop and average respiration rate over a two-hour period. This time interval was used in order to correct for night time respiration.

Crops ranging from 5 to 10 mg chl/m³ had the highest average rate of net photosynthesis. This is significant in view of the previous discussion in which it was pointed out that the most frequently occurring populations had chlorophyll contents within this range (Table 5) and would be most likely to be in a steady state condition. This may indicate that there is a tendency for standing crops of phytoplankton to become adjusted to a level which will deliver maximum net production, as Odum and Pinkerton (1955) have postulated. The smaller crops which were the most efficient per unit chlorophyll delivered a smaller net

production than did the larger crops which were only about 50 % as efficient.

It will also be noted that a wide range of crops smaller and larger than the optimal size crop had nearly the same net photosynthetic rate as the optimal size crop. After crops became larger than 20 mg chl/m³, the rate of net photosynthesis rapidly decreased. The largest crop encountered in the study (70.6 mg chl/m³) had a negative rate of net photosynthesis and rapidly declined. The plankton at this time was predominantly composed of *Aphanizomenon flos-aquae*, which had rapidly built up to bloom proportions from a level of 1.7 mg chl/m³ five days previously. The rapid increase followed a mass mortality of *Daphnia* and a great increase in soluble phosphate and was partially the result of concentration by advection in the vicinity of the sampling station.

Objection might be raised against the foregoing argument on the grounds that the limiting effects of nutrient deficiency were ignored. On the basis of culture experiments, Ryther (1956) found that the photosynthesis:respiration ratio approached unity after nutrients were depleted and growth had stopped. However, growth of algae in culture experiments of this type is not equivalent to the growth of algae in lakes. In a natural situation the observed rate of growth is a balance between actual rate of growth and rate of removal by grazing, currents, sinking, etc. The natural situation is more comparable to the continuous flow culture experiments carried out on *Chlorella* by Cook (1950). In his experiments, population density was kept constant by adjusting the rate of overflow from the culture. Hence the growth rate and the yield (growth rate × density) could be determined from the rate of overflow. It was found that growth rate decreased with increase in density as would be expected. However, yield reached a maximum value of 0.48 g/L/day at a population density 0.36 g/L. Population densities above or below 0.36 g/L gave lower yields. Yield, as used by Cook, is equivalent to net photosynthesis in Table 12, if the values in Table 12 were converted to dry matter equivalents of net photosynthetic oxygen production. On this basis net production in relation to standing crop (density) behaved similarly in the laboratory experiment and in the lake.

The major difference is that environmental factors affecting growth and removal rates vary in a natural situation in contrast to a laboratory experiment where they can be controlled. In discussing the results in Table 12 it was implied that during the period of time the samples were collected, an average set of conditions existed, and that within the usual range of these conditions an optimal size population could be defined as one giving maximum net production.

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A Mechanism for the Mixing of the Waters of Lake Huron and South Bay, Manitoulin Island¹

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ABSTRACT

The seiche in South Bay, Manitoulin Island, Ontario, consists of several components, some of which produce considerable displacements of the water which occupies the narrow entrance channel. Lateral currents displace Lake Huron water intruded into the bay by the seiche excursion, so that the return portion of the seiche cycle replaces the intruded water with Bay water, the exchange providing the mixing process. The seiche amplitude appears to be the major variable controlling the intensity of the mixing process.

Part 1—THE SEICHE IN SOUTH BAY

The Experiment:—During the first week of August 1952 three water level recorders were installed in South Bay, Manitoulin Island, Ontario. One was located in the Small-Boat Harbor at South Bay Mouth. Another was installed on Sims Island, and the third was near the John S. Pheasant home at the northeastern end of South Bay (Fig. 1). The Leupold-Stevens Type F recorders were fitted with gears to give 25 cm of record length at full scale per 24 hours. These were mounted on 32-gallon stilling wells with $\frac{3}{8}$ -inch orifices.

On August 4 and 5 there was a storm with strong southwesterly and westerly winds, which abated on August 5. This storm developed a pronounced seiche of several component frequencies which slowly decayed over the next few days.

Records:—A portion of this seiche record is shown in Figure 2. All three observations showed a general rise as the high wind stress set up Lake Huron. Superimposed on this general rise was a long oscillation at about six-hours period, synchronous at all points. Rough computations by Merian's formula show that this must be an extension at the Lake Huron Seiche, as suggested also by the uniform time of cresting. This then was a co-oscillation.

Several other oscillations were also apparent. There was a nine-minute oscillation evident throughout the record, which may represent a transverse oscillation in the bay. During the early part of the record a 53-minute period was present, which gave way to a 38.9-minute period on the evening of August 6. These shorter period seiches must be oscillations of the bay water mass, either forced or free.

The record from Sims Island is unusual in that while the very short and very long period seiches were present, the intermediate period oscillations were nearly absent. The most likely explanation is that the intermediate period waves may rotate around the bay with an amphidromic point on Sims Island. If the recorder were faulty it would not be likely that the very short period would be present.

Analysis:—Step-wise integration of the equation of continuity was performed for the South Bay basin along the lines laid down by Sterneck and by Defant (described by Sverdrup *et al.* 1946). In this case, where the period is known, the method yields an estimate of the water surface configuration in the bay, and in particular sheds light on what the relationship to Lake Huron might be. Essentially, the method is based on the condition that there can be no horizontal motion through the closed end of the bay, plus the assumption that the local change of water velocity associated with the seiche movement is produced by the pressure gradients due to the slope of the lake surface. Thus, knowing the seiche

¹ The authors wish to recognize the kind assistance of Dr. F. E. J. Fry and Mr. John Budd who made possible the field work described in this paper, under the auspices of the Ontario Department of Lands and Forests, at the South Bay Laboratory.

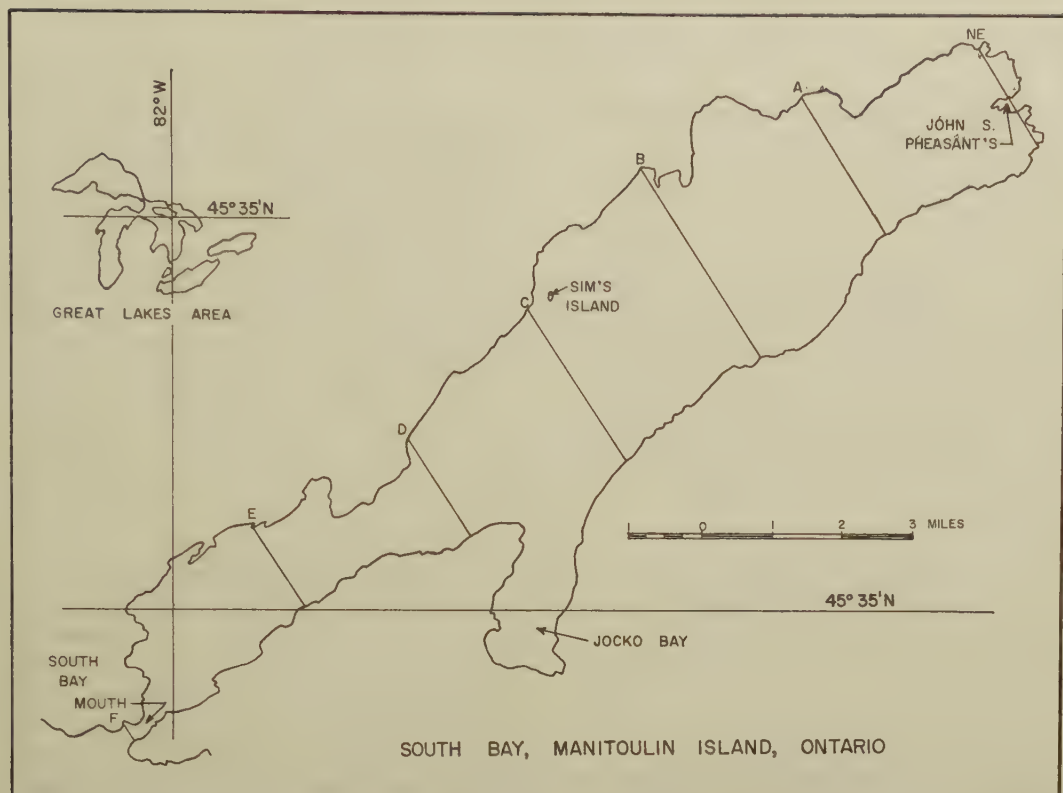


FIG. 1. Guide map to locations about South Bay, Manitoulin Island, Ontario. Lettered Sections indicate location of sections used in calculations. The inset map shows the location of Manitoulin Island at the north end of Lake Huron; the latitude-longitude intersection on the small map is the same as that indicated on the map of South Bay.

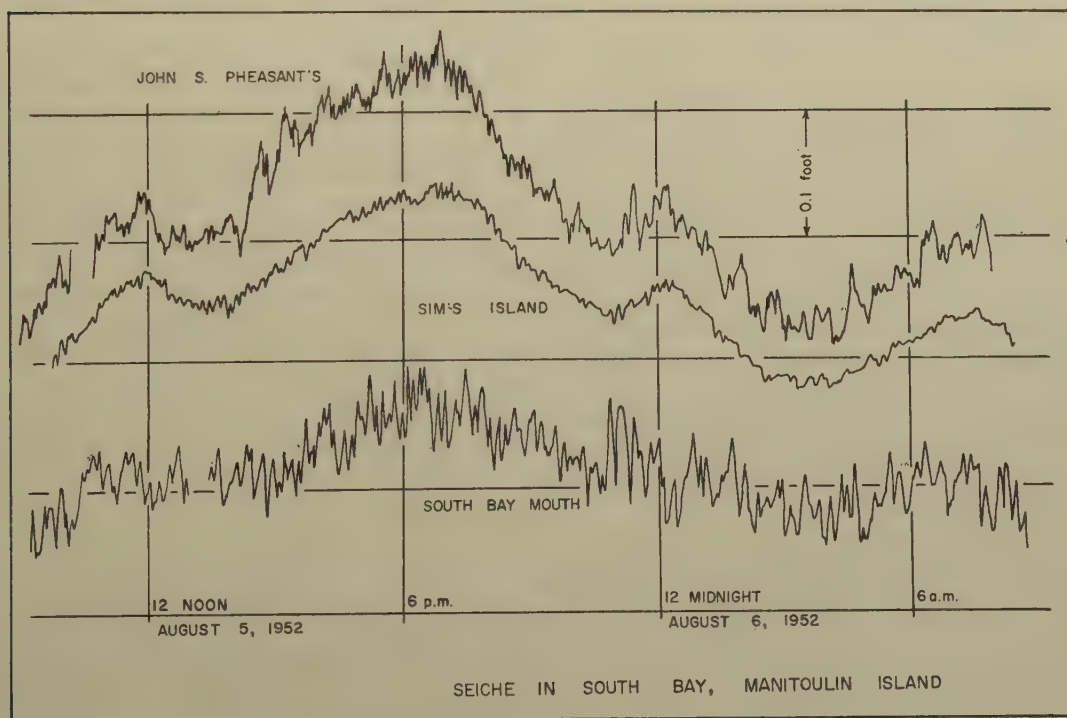


FIG. 2. A portion of the seiche record for three South Bay locations, August 1952.

TABLE 1. Vertical and horizontal displacements at selected sections

	9 min period		38.9 min period		53 min period	
	h	v	h	v	h	v
NE	000	1.00	000	1.00	000	1.00
A	-65	0.83	-388	0.95	-393	0.97
B	-1865	0.19	-255	0.86	-264	0.92
C	-135	-0.65	-352	0.77	-372	0.88
D	-9	-1.02	-900	0.60	-891	0.78
E	+289	-0.29	-4110	-0.09	-464	0.68
F	+174	+1.07	-16850	-2.99	-24400	-1.43
G	-109	+1.55	+139	-5.31	-202	-3.36

h = horizontal displacement in cm.
v = vertical displacement in cm.

TABLE 2. Calculated properties of the South Bay seiche assuming an amplitude of 1.0 cm

Component period	Amplitude at Bay Mouth	Displacement at Bay Mouth	Mean velocity*	Nodes
9 min. (a)	1.100	283.0	2.1 cm/sec	2
(b)	1.066	174.3	1.3 cm/sec	2
38.9 min.	2.993	16850.0	28.9 cm/sec	1
53 min.	1.425	24400.0	30.7 cm/sec	1
61.9 min.	0.859	32730.0	35.3 cm/sec	0

(a) Jocko Bay included.
(b) Jocko Bay omitted.

* i.e., mean velocity through the channel at South Bay Mouth.

period, the shape of the basin, and the amplitude of the seiche at the closed end of the bay, the rate at which water must flow into or out of that section of the bay may be computed. However, this rate determines the slope of the lake surface in that portion of the bay, hence the displacement of the next section etc. In this way, the vertical displacement of the lake or bay surface may be computed along the length of the basin. In the case of a bay, such as South Bay the calculation yields a measure of the displacements, horizontal and vertical, at the mouth of the bay for each observed seiche period.

The horizontal and vertical displacements obtained by using periods of 9, 38.9, and 53 minutes and assuming a terminal amplitude of one centimeter are given in Table 1. Location of the sections indicated in the table are shown in Figure 1.

During one complete seiche period a parcel of water must move four times the maximum displacement in one direction. For example, if the maximum displacement in one direction from the average or null position is 100 meters, then during the course of one seiche period the water must move 100 meters one way, return to the null position, then do the same in the opposite direction for a total movement of 400 meters. Dividing the total movement by the seiche period yields an equivalent average velocity. This has been done for the narrow entrance to South Bay, and the results are shown in Table 2.

Discussion:—It is generally assumed that a bay seiche must be such that a node is located across the mouth of a bay (Sverdrup *et al.* 1946). This is but partially realized in the case of South Bay, for the Small-Boat Harbor record was certainly not free of the seiches found inside South Bay. The calculations indicated that the 53-minute seiche should have been relatively small there, and this was true. The calculated amplitude of the 53-minute seiche at South Bay Mouth is 43% larger than at John Pheasant's and was observed to be about 30% larger, possibly due to the friction in the narrow channel.

The 39-minute period that was evident later in the record presents several complications. It was present with an amplitude of 0.03 feet at John Pheasant's but was reduced to 0.01 feet at Sims Island, and was of opposite phase. At the bay mouth this oscillation was replaced by one of 78-minutes period, i.e., the first harmonic.

It would be most interesting to attempt an evaluation of the bottom stress from the rate of decrease of seiche amplitude (Bryson and Kuhn 1955), then evaluate the period lengthening due to friction (Defant 1932). This would require more complete field observations than are available, however.

Part 2—MIXING OF HURON AND SOUTH BAY WATER

The Problem:—Utilizing the results of the preceding analysis we may now consider the problem of mixing through the South Bay Mouth channel. Temperature profiles of

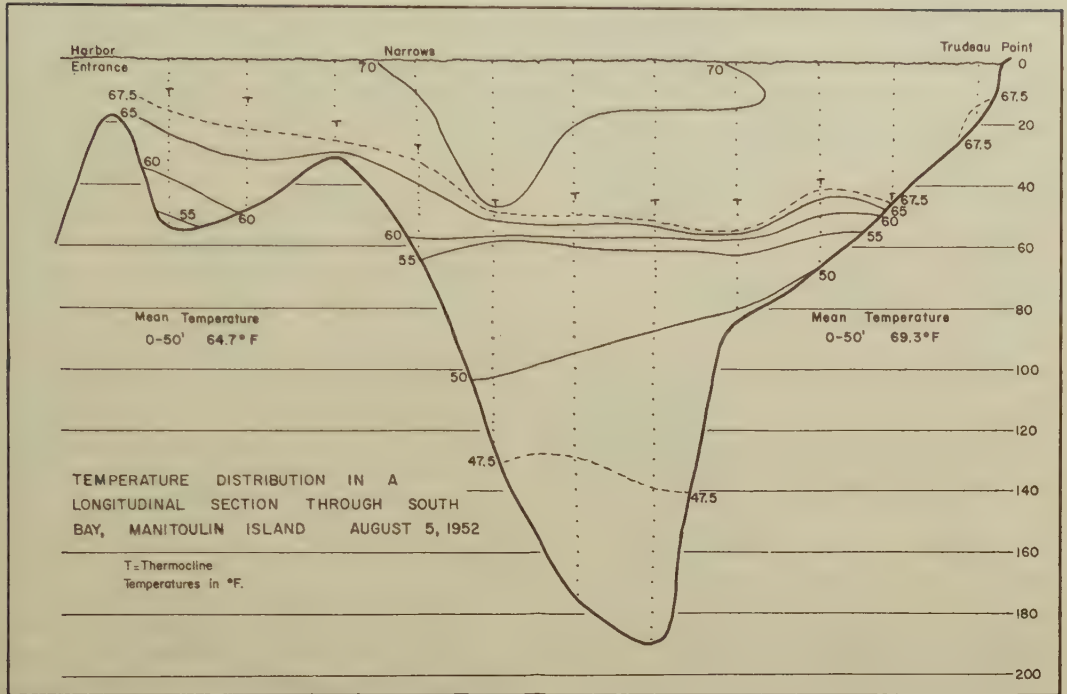


FIG. 3. Temperature profile through the length of South Bay, August 5, 1952.

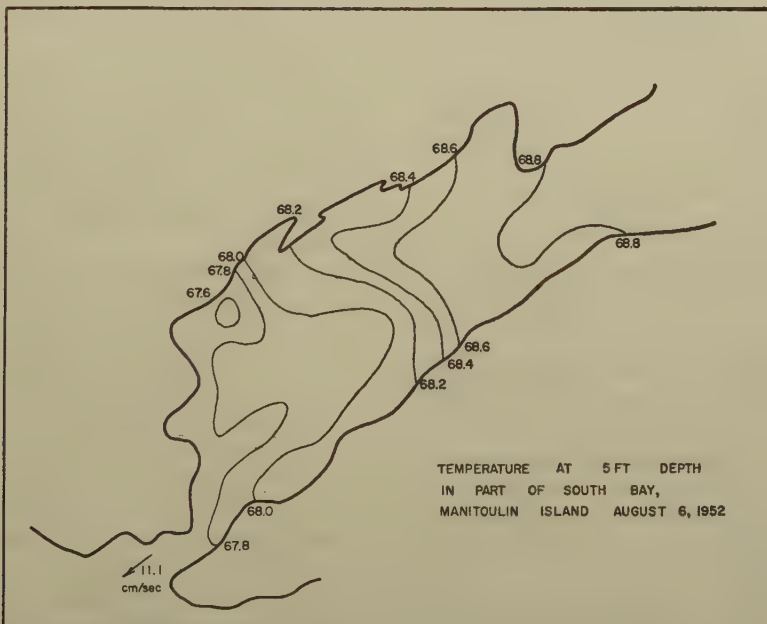


FIG. 4. Distribution of temperature at 5 feet depth in a portion of South Bay, August 6, 1952.

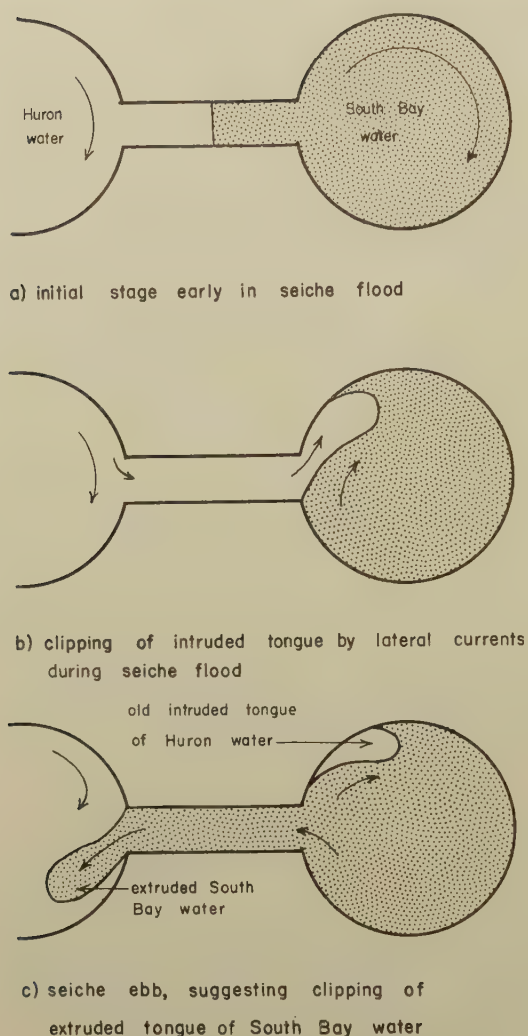


FIG. 5. Schematic illustration of the mode of mixing of South Bay and Lake Huron water through South Bay Mouth Channel.

South Bay suggest that the smaller basin, which separates the main portion of South Bay from Lake Huron, is occupied by perhaps one-third Huron water and two-thirds South Bay water (Fig. 3). Considering the wide opening between the two basins of South Bay and the comparatively tiny opening into Lake Huron it is, at first glance, rather surprising that there is any appreciable admixture noticeable in the southwestern basin.

A clue to the answer is to be found in the results of a temperature reconnaissance of

the southwestern basin made on August 6, 1952 (Fig. 4). The only unusual feature of this temperature distribution is the irregular streak of cold water, suggestive of Lake Huron temperatures, to be found just inside South Bay. Turning back to the computed displacements for a one-centimeter amplitude seiche (Table 1) it appears that during each cycle of the seiche a 200–400 meter long tongue of water must be intruded into South Bay from the entrance channel. On the opposite swing of the seiche cycle a similar tongue must be extruded into Lake Huron. If the horizontal currents are sufficient to displace these intruded and extruded tongues the width of the channel in one-half seiche cycle, then the water extruded into Lake Huron will be South Bay water, while that intruded into South Bay will be Huron water.

Figure 5 illustrates schematically the mode of mixing. Breaking the sequence of events into steps—which of course is not the case in nature—we have, during the first half of the seiche cycle, intrusion of Huron water through the South Bay Mouth channel. Next the horizontal currents normal to the bay mouth channel in South Bay displace the tongue of Huron water laterally so that on the reverse swing of the seiche South Bay water is extruded into Lake Huron. That portion of the water that remains in the channel at the crest of the seiche must of course be returned to the region of its source.

Looking at this mechanism quantitatively, we may let

- S = seiche amplitude (cm),
- E = displacement of water through the bay mouth, *i.e.*, the seiche excursion, per unit seiche amplitude,
- A = cross-sectional area of the bay mouth channel (cm²),
- T = seiche period (min.),
- V = volume of the bay (cm³),
- C = channel length (cm).

Then it is clear that the total amount of exterior water intruded into the bay per seiche cycle is given by the product $(SE - C)A$. Dividing by the seiche period and the volume of the bay gives the rate of admixture of exterior and interior water per unit time. Thus

$$\text{percentage rate of admixture per day} = \frac{(SE - C)A}{TV} \times 1.44 \times 10^5$$

if the seiche period is measured in minutes. If the length of the entrance channel is greater than the seiche excursion, $(SE - C)$ in the above expression becomes negative, signifying no mixing by this mechanism. If the channel is broad, not only is the excursion generally reduced, but the horizontal currents will possibly be too weak for complete displacement of the intruded water, and the rate of admixture would be proportionately reduced. The exact expression for the amount of reduction would of necessity be complicated, but an order of magnitude of the reduction factor would probably be given by the fraction TL/W , where

T = seiche period in minutes,

L = lateral current velocity in centimeters per minute,

W = width of channel in centimeters.

The reader will have noted that in the expression developed the *only variable* for a given bay, providing the assumed conditions are met, is the *seiche amplitude*.

Assuming an effective channel length of

400 meters and a seiche amplitude of 2 cm, along with the other dimensions of the southwestern basin of South Bay, gives a calculated rate of mixing on the order of 10% per day. This is a high rate, but this particular seiche amplitude followed a rather strong storm, and the effective channel length may be greater. The dimensions of the cold intruded tongue in Figure 4 suggest that this 10% figure may be the correct order of magnitude for August 6. At the same time the calculated seiche excursion through the Narrows (separating the two main basins) was considerably less than the length of the Narrows channel. It is not strange then that the southwestern basin should have such a large admixture of Huron water.

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Some Relations Between Air Temperatures and the Surface Water Temperatures of Lakes

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ABSTRACT

An analysis of meteorological and hydrographic records collected at South Bay, Ontario, over the past nine years shows that when the monthly mean surface water temperatures for successive years are plotted against the monthly mean air temperatures, the general relation can be described by two linear regressions. One of these lines corresponds to the May-July period in which the temperatures rise, while the other covers the August-November period in which they fall. A quite comparable picture is also obtained for Lake Opeongo, Ontario, and Lake Mendota, Wisconsin. The latter bodies differ markedly from South Bay in morphometry and location. Lakes Opeongo and Mendota have continental situations, whereas South Bay is enclosed in a comparatively small land mass which in turn is surrounded by very large water masses.

INTRODUCTION

From time to time attempts are made to correlate the year class strength of fish populations with the weather conditions prevailing during the spawning, hatching, and early growth. Unfortunately the investigators are often handicapped by an inadequacy of water temperature data and are obliged to take the air temperatures from standard meteorological reports as approximations of the hydrographic conditions. Therefore, it is worthwhile to look for relations between the air and water temperatures which will enable one to make these approximations more accurate.

One such relation has been described by Sette (1940). From records of air and water temperatures collected at Lake Meade in Nevada during 1938 he demonstrated that the daily rate of change in water temperature was a linear function of the difference between the air and water temperatures. He then showed how this relation could be used to predict water temperatures from the monthly means of the air temperatures, if one assumed to begin with that the mean water temperature for March would be about 13°C. In his demonstration he was able to make predictions of the surface water temperatures to be expected in the Shasta reservoir when the dam was built which proved to be surprisingly close to those observed later (Rawson 1958).

Another relation between air and water temperatures is described by Fry and Watt (1955) in a recent study of the effects of weather on the bass populations in South Bay and neighbouring waters in Manitoulin Island. These workers show that, for each month from June to October, there is a linear relation between the thermal sum for the surface waters and the monthly mean of the air temperatures. They also show that there is a linear relation between the year class strength of the bass in South Bay and the year to year deviations from the monthly mean air temperatures, when these deviations are summed over the period from June to October.

In the present paper the possibility of predicting water temperatures from air temperatures is pursued further with respect to the data from South Bay. In addition, this work deals with the relation between air and surface water temperatures at Lake Opeongo in Algonquin Park, Ontario, and at Lake Mendota in Wisconsin. The continental locations of these two lakes contrast with that of South Bay. The latter body has a maritime situation in the sense that it is enclosed by a comparatively small land mass, which in turn is surrounded by large water masses. It is conceivable that such a maritime situation might favor the close correlation between water and air temperatures found at South Bay, but it is surprising

that the relation is essentially the same for the continental lakes.

The writer is much indebted to F. E. J. Fry and L. Butler of the Department of Zoology, University of Toronto, for advice on the statistical treatment of the data in this paper. Many thanks are also due to N. V. Martin of the Ontario Department of Lands and Forests for making available the data from Lake Opeongo.

LOCATION AND MORPHOMETRY OF BODIES OF WATER

South Bay extends from Lake Huron into the south-eastern corner of Manitoulin Island, which in turn is situated in the second largest water mass in the Great Lakes System. The bay is about 16 miles long by three miles across at the widest point and has an area of 31 square miles (8,000 hectares). It is connected with the lake by a passage 700 feet wide. At about one-third of the distance from the mouth to the upper end of the bay there is a narrows 0.3 miles in width, which divides it into two basins (Fraser 1955). The maximum depth of the outer basin is about 60 feet (18 meters) and that of the inner basin 200 feet (60 meters).

Lake Opeongo lies near the southern edge of the Precambrian Shield about 90 miles east of Georgian Bay and 120 miles north of Lake Ontario, the nearest links in the Great Lakes chain. To the north lies the continental mass of northern Ontario and Quebec. The total area of Lake Opeongo is approximately 20 square miles (5,200 hectares), and the maximum depth is 175 feet (58 meters) (Fry 1949). The area comprises a large north arm, somewhat smaller east and south arms, and Sproule Bay, which is a lobe off the south arm.

Lake Mendota is located about 75 miles west of Lake Michigan and 250 miles south of Lake Superior. To the west stretches the great continental mass of central North America. Lake Mendota is roughly heart-shaped and has an area of approximately 15 square miles (3,900 hectares) and a maximum depth of 84 feet (26 meters) (Juday 1914).

NATURE OF THE TEMPERATURE RECORDS

The temperature data in this work are given as monthly means because that is the

most readily available unit for studies on Canadian waters. It is the unit published by the Meteorological Branch of the Department of Transport. The quadri-monthly means given by Neess and Bunge (1956) for Lake Mendota, Wisconsin, have been converted to monthly means in order that they may be compared with the data for South Bay and Lake Opeongo. However, the smaller unit would probably permit a more exact determination of the relation between air and water temperatures and would be preferable for studies in which a comparison between lakes was not desired.

The meteorological and hydrographic data for South Bay were collected at the Ontario Department of Lands and Forests, Division of Research, Fisheries Station on the north-west shore of the outer basin. The monthly means of the air temperatures are calculated from the daily maxima and minima, which have been kept at the station since 1949. The monthly mean water temperatures for the years 1949 to 1953 are based on readings of the daily maximum and minimum made from the station wharf about 50 feet from shore in from three to five feet of water. For the years 1954 to 1958 the mean monthly water temperatures are based on bathythermograph readings made at a position 300 feet off the point of the station wharf at intervals of a week to ten days. Bathythermograph readings made at positions on the outer and inner basins on the same day at regular intervals throughout the open seasons of 1954 to 1957 show that the surface water temperatures are approximately the same in the two basins.

The monthly means of the surface water temperatures for Lake Opeongo are calculated from readings made at the Ontario Department of Lands and Forests Fisheries, Division of Research, Laboratory on the east shore of Sproule Bay. These readings were made with a thermograph, the bulb of which was immersed in about four feet of water in the well of a boathouse. Records collected at scattered intervals on the north, south, and east arms of the lake suggest that although the daily readings of the surface water temperatures in those basins sometimes differ by one or two degrees, the monthly averages are probably close to the

monthly averages for Sproule Bay. The corresponding air temperature data were obtained from records of the daily maximum and minimum observed at the Algonquin Park Headquarters. The latter station is about 16 miles west of Lake Opeongo.

The temperatures of the air and surface water at Lake Mendota given herein are derived from graphs in the manuscript by Juday, which was recently published by Neess and Bunge (1956). The graphs referred to present plots of quadri-monthly means of the air and water temperatures against time for each of several years between 1895 and 1916. They illustrate primarily the correlation between the changes which take place in the air and the water temperatures from one season to another. In the present paper, on the other hand, the mean temperatures have been recalculated on a monthly basis for comparison with the South Bay and Lake Opeongo data, and the mean surface water temperature has been plotted against the mean air temperature in such a way as to emphasize the correlation between the changes which occur in these quantities from year to year.

The water temperatures in the publication by Neess and Bunge are based on readings made by Juday at two fixed points on the open waters of Lake Mendota with an electrical resistance thermometer. The corresponding air temperatures were taken from records gathered during the same period at the United States Weather Bureau Station near the shore of Lake Mendota at Madison.

OBSERVATIONS

Figure 1 shows the relation between the monthly means of the surface water temperatures and air temperatures at South Bay. Each cluster of points represents the data for a particular month which were collected in the years 1949 to 1958. The smaller numbers of points for April and November are due to the fact that records were not made for these months in all years. A linear regression line has been fitted to the points for each month by the method of least squares, and the slopes and intercepts of these lines are given in the third and

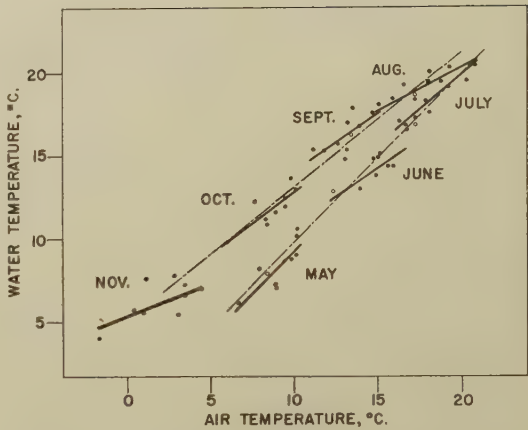


FIG. 1. The relation between the monthly means of the air and surface water temperature at South Bay. The solid lines represent the regressions for individual months. The broken lines are fitted to the 1958 data, denoted by the open circles, to show the general trends of the warming and cooling phases in the relation.

TABLE 1. The slopes and intercepts of the monthly regression lines shown in Figures 1, 2, and 3, and the average deviations of the predicted mean water temperatures from the observed means

(1) Location	(2) Month	(3) Slope	(4) Intercept °C.	(5) Av. Dev. ¹ °C	(6) Av. Dev. ² °C
South Bay	May	1.02	-19.0	0.7	0.8
	June	0.64	-7.0	0.5	0.7
	July	0.82	-11.5	0.5	0.6
	Aug.	0.54	0.0	0.4	0.7
	Sept.	0.71	-5.7	0.6	0.9
	Oct.	0.75	-8.1	0.5	0.6
	Nov.	0.43	-2.3	0.7	1.0
Lake Opeongo	May	0.74	-10.0	0.2	
	June	0.29	6.8	0.6	
	July	0.92	-12.2	0.2	
	Aug.	0.70	-4.0	0.3	
	Sept.	0.50	0.7	0.6	
	Oct.	0.51	-2.3	0.2	
Lake Mendota	April	0.53	-0.7	1.5	
	May	0.90	-0.5	0.6	
	June	1.10	-1.8	1.1	
	July	0.81	-10.4	0.8	
	Aug.	0.64	9.7	0.9	
	Sept.	0.62	9.4	0.6	
	Oct.	0.70	7.0	0.8	
	Nov.	0.80	5.8	0.8	

¹ For temperatures predicted from monthly regressions.

² For temperatures predicted from general regressions.

fourth columns of Table 1. The average deviations of the points from the regression lines are given in the fifth column of the table.

It is evident that there is a close correlation between the monthly means of the surface water and air temperatures at South Bay, and that the general relation between these quantities may be described as a linear regression of surface water temperature on air temperature. However, different phases can be recognized in this general relation when the regression lines for the individual months are compared. One of these phases includes May, June, and July, the months in which the water and air warm up. The slopes of the regression lines for May and July, which are 1.0 and 0.8, respectively, indicate that for these months the variation which takes place in the water temperature from year to year is almost the same as that which occurs in the air temperature. On the other hand, the slope for June is 0.6, which shows that for that month the year to year variation in water temperature tends to be less than the variation in air temperature. It may be that this smaller variation in water temperature for June is related to the fact that thermal stratification is finally established in South Bay during that month.

August, the month in which the water and air temperatures reach their maxima at South Bay, seems to form a transition phase from warming to cooling. In that particular month the slope of the regression line is low, namely 0.5.

A third phase in the relation between the South Bay air and surface water temperatures occurs in September, October, and November, the months in which the air and water cool down. The fact that the regression lines for this third phase have lower slopes and considerably higher intercepts with the water temperature axis than do the lines for May and July indicates that the water temperature has less year to year variation than air temperature during the cooling phase. This smaller temperature variation probably reflects the higher specific heat and consequent greater heat retention of the water.

The points for November in Figure 1

show a greater scatter than do the points for the preceding months, and the regression between the water and air temperatures is much less marked for that month. It is possible that this greater scatter is associated with the final breakdown in thermal stratification which takes place in South Bay during November.

A fourth phase in the relation between the water and air temperatures at South Bay is not shown in Figure 1. This phase extends from December to mid-April and includes the period of ice-cover. There may be a considerable variation in the monthly mean air temperatures in this last phase, but the mean surface water temperature remains slightly above 32°F from one year to another.

Insofar as the upper basin of South Bay is concerned, the regression lines described above can be used to predict not only the surface water temperature but also the temperature at any depth down to the twenty-foot level. Bathythermograph readings were made at seven stations on that basin at intervals of one or two weeks from May to November during the years 1954 to 1957. These showed that the temperatures from the surface to the twenty-foot level did not differ by more than two degrees centigrade. Moreover, in the majority of readings the difference was less than one degree. When thermal stratification is established in South Bay the lower boundary of the epilimnion corresponds approximately to the twenty-foot level.

Simultaneous bathythermograph readings made at five stations on the lower basin of South Bay indicate that the temperature in that part also tends to be uniform from the surface to the twenty-foot level. However, this tendency to uniformity is frequently distorted during the summer months by masses of cold water which move into the bottom of the lower basin from Lake Huron as a result of seiches. This exchange of water masses will be dealt with in a more detailed description of the hydrography of South Bay which is now in preparation. At this point it is sufficient to recognize that this factor restricts the prediction of water

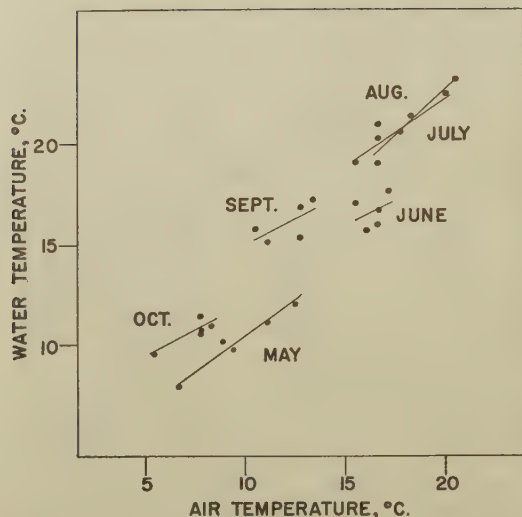


FIG. 2. The relation between the monthly means of the air and surface water temperatures at Lake Opeongo.

temperatures to a shallower layer in the lower basin than in the upper.

Figure 2 illustrates the relation between the monthly means of the air and surface water temperatures at Lake Opeongo. The picture is similar to that given in Figure 1 for South Bay, although the data are more fragmentary. May, June, and July belong to the phase of warming. The slope of the regression line for June, the month in which thermal stratification is established, cannot be accurately determined on the basis of the data available. There is also a cooling phase in which the regression lines tend to have lower slopes and higher intercepts with the water temperature axis than do the lines for the warming phase. As pointed out above, these higher intercepts indicate that there is a smaller year to year variation in the water temperature than in the air temperature, owing to the higher thermal capacity of the former medium. In Lake Opeongo this cooling phase seems to include

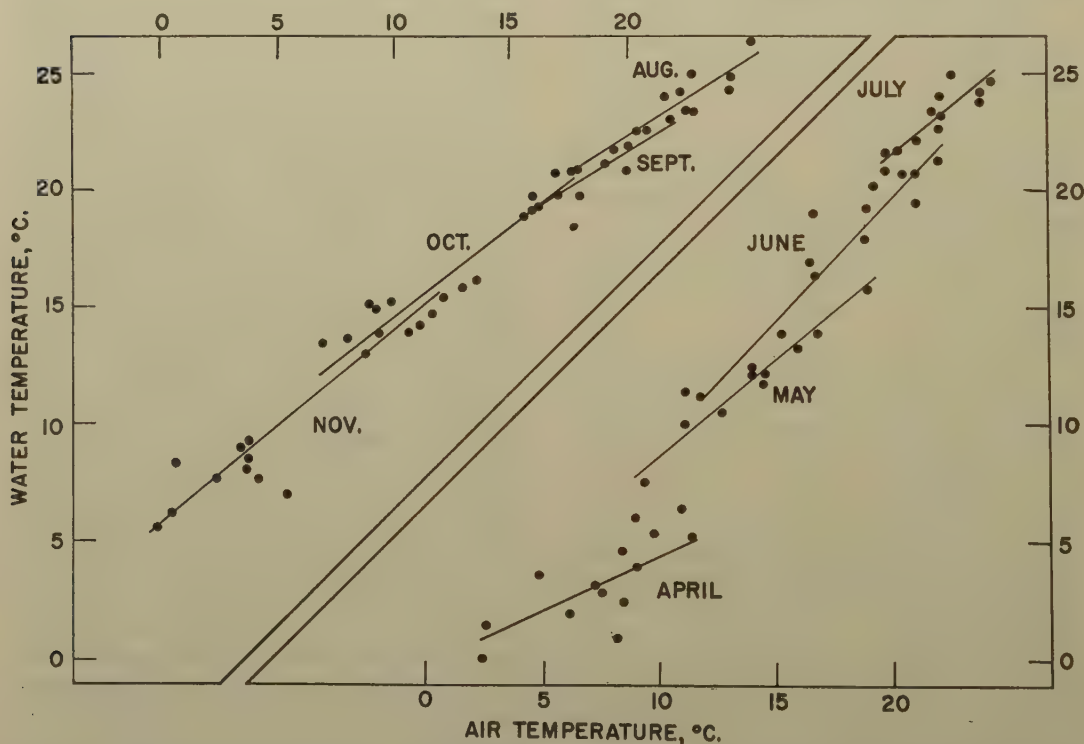


FIG. 3. The relation between the monthly means of the air and surface water temperatures at Lake Mendota. The warming and cooling phases of the relation are plotted separately because of the marked overlap in the data for July, August, and September.

August as well as September and October. Adequate data are not available to permit a discussion of the period from November to April. The slopes and intercepts of the individual regression lines and the corresponding average deviations of the points from these lines are listed in Table 1.

Figure 3 shows the relation between the monthly means of the air and surface water temperatures at Lake Mendota. This figure has been plotted in two sections because the points for July, August, and September overlap to such an extent that they would be difficult to distinguish if they were plotted together. The slopes and intercepts of the regression lines and the average deviations of the points are again given in Table 1.

Figure 3 shows that the relation between the temperatures of the air and surface water for Lake Mendota is essentially the same as that for South Bay and Lake Opeongo. May, June, and July belong to a warming phase in which the slopes of the regression lines range from 0.8 to 1.1. August, September, October, and November constitute a cooling phase in which the slopes range from 0.6 to 0.8 and the y-intercepts are from 8 to 10 degrees centigrade higher than the corresponding intercepts for the warming phase. April marks a transition from a winter phase to the phase of warming. However, there are two respects in which the relation between air and surface water temperatures for Lake Mendota differs from that of South Bay and Lake Opeongo. One difference is that there is a much greater overlap in the points for successive months for Lake Mendota than for the other bodies. Although this greater overlap may be due in part to there being more data for Lake Mendota, it also suggests that both the air and water temperatures vary much more from year to year at Lake Mendota than at the other two locations. Therefore, the location of Lake Mendota may be even more truly continental than that of Lake Opeongo.

The other respect in which the relation between air and water temperatures for Lake Mendota differs from that of South Bay and Lake Opeongo is that the slope of the June regression line for Mendota is not

markedly reduced as it is for the other two. On the contrary, the slope of the June regression line for Lake Mendota is greater than the slope for any other month (Table 1). The reason for this difference in temperature relations is not apparent at present.

COMMENT

It is evident that the relation between the monthly means of the air and surface water temperatures may differ from one month to another. Hence the estimation of surface water temperatures from air temperatures will be most reliable when there are sufficient data available to permit the construction of a regression line for each month. An example of the agreement between the water temperatures predicted from such regressions and the observed values is given in Figure 4. In all but two instances the deviation of the prediction from the observation is less than 1.0°C and at most is less than 1.5°C (see Table 1, column 5).

However, an examination of Figures 1, 2, and 3 suggests that if the temperatures of the surface waters were available for even one year, two general regression lines could be drawn from which the water temperatures for other years could be estimated within

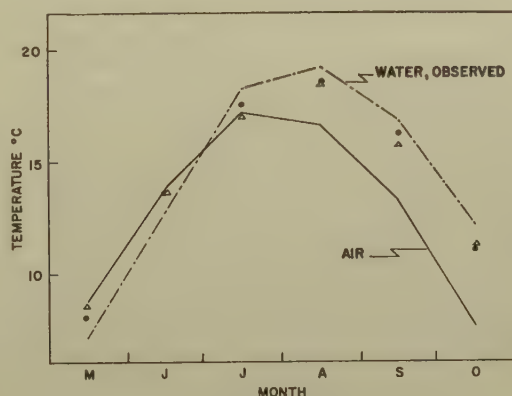


FIG. 4. A comparison between the 1958 temperature data for South Bay. The solid line connects the monthly means of the air temperatures while the broken line links the monthly means of the observed surface water temperatures. The dots represent surface temperatures predicted from the monthly regression lines in Figure 1. The triangles represent surface temperatures derived from general trend lines in Figure 1.

fairly narrow limits. One of these lines would be fitted to the data for May, June, and July to show the trend of the warming phase in the relation between air and water temperatures. The other line would be fitted to the data for August, September and October to describe the cooling phase. The predictions would be made by taking the monthly mean air temperatures from the meteorological records for the years in question and finding the corresponding surface temperatures from these lines. A pair of such lines has been fitted to the 1958 data in Figure 1, and the temperatures predicted from them for that year are compared with the observed values in Figure 4. Although the average deviations of temperatures estimated from the general lines are likely to be greater than those for temperatures derived from monthly regressions, they will probably be less than two degrees centigrade (see Table 1, column 6).

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Regional and Seasonal Variation in the Respiratory Rate of Marine Copepods¹

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ABSTRACT

The respiratory rate of seven species of marine calanoid copepods was measured at the same temperature, and an attempt was made to find a basis for comparison of respiration in zooplankton crustaceans of different size. When the log of the respiratory rate was plotted against either log length or log dry weight, a positive linear correlation was obtained, but in each case the correlation coefficient was greater than would be expected if metabolism were related to surface area. When the respiratory rate was plotted against dry weight without logarithmic transformation, a linear correlation coefficient $r = 0.978$ was obtained. When dry weight was used to correct for size differences between populations, *Acartia clausi* from Southampton water had a statistically higher metabolic rate in laboratory experiments during the cooler months of the year than those from Long Island Sound at the same season. Seasonal variation in metabolism independent of temperature was demonstrated for several species of copepods, and some of the factors that might be responsible for this variation are discussed.

There is appreciable evidence to suggest that marine poikilotherms can exert some control over their metabolism independent of environmental conditions. The subject of temperature regulation has been reviewed by Bullock (1955). Presumably, marine copepods are also able to acclimate within limits to prevailing habitat conditions, and as a result regional and seasonal variations in metabolism should be expected. No experiments with a view to demonstrating such differences have been performed to date, and comparison of results in the literature is complicated because few authors have made an attempt to relate metabolism to body dimension or mass. The respiration of *Calanus finmarchicus* appears to be about 50% greater in waters off Woods Hole, Massachusetts, than in the Clyde Sea area (cf. Clarke and Bonnet 1939; Marshall *et al.* 1935), but no correction for possible size differences between the two populations can be made. Gauld and Raymont (1953) who studied neritic copepods of Southampton Water and Conover (1956) who studied species of the genus *Acartia* in Long Island

Sound (hereafter referred to as L.I.S.), each present some evidence to suggest that respiration may vary from generation to generation or season to season. The one species studied from both environments, *Acartia clausi*, appeared to have a respiration rate up to 100% greater in British waters than in Long Island Sound although again comparison per metabolic unit was not easily accomplished.

Because of the importance of copepods in the transfer of energy in the aquatic food chain, it is desirable to know more about their metabolism under different conditions of existence and to be able to relate this information quantitatively to other environments. In this paper, respiratory measurements are extended to include several more species of neritic copepods. In addition an attempt is made to establish a reasonable basis for comparing species studied at different seasons or from different locations.

The author would like to thank Gordon A. Riley for his helpful suggestions in the L.I.S. work and Professor J. E. G. Raymont for his assistance and encouragement during the work undertaken in England. In addition, the author wishes to express his gratitude to the United States Educational Commission in the United Kingdom which pro-

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vided the opportunity to visit England through a Fulbright award.

MATERIAL AND METHODS

Copepods used in all experiments were captured with a No. 2 bolting cloth net or in England with a "medium" plankton net of similar size mesh. After capture the samples were suitably diluted and brought to the laboratory where they were kept over night in a cold room at a temperature between 5–10°C before use. In England the animals were usually given a quantity of *Dunaliella* to supplement the natural food in the water. This was not considered necessary in the richer waters of L.I.S. Animals to be used for experiments were picked out of stock cultures with the assistance of a low-power microscope and transferred to filtered sea water. (In the British experiments, Plymouth "outside" sea water was used.) Seven species were studied, although availability necessarily limited the number of experiments for each. The species used were *Acartia clausi* (Giesbr.), *A. tonsa* Dana, *A. discaudata* (Giesbr.), *A. bifilosa* (Giesbr.), *Centropages hamatus* (Lilljeborg), *Temora longicornis* (Müller), and *Pseudocalanus minutus* (Krøyer).³

Two methods were employed to measure copepod respiration. The modified Winkler method described by Conover (1956) was used in all L.I.S. studies. The standard error of titration could be made less than 0.02 ml oxygen/liter, and replicate experiments generally were in good agreement, but the method does have the disadvantage that oxygen utilization can be determined over only a single time interval in any one experiment. In England, this method was supplemented with measurements obtained with the Barcroft-Dixon constant pressure manometers as used by Raymont and Gauld (1951) and Gauld and Raymont (1953). It was found that the variability

in manometric experiments was great unless each manometer was read at the same room temperature during the course of an experiment. The entire water bath was kept in a cold room at 9–10°C, and the water within the bath was maintained at $20 \pm 1^\circ$. There was no statistically significant difference between the results obtained with the two methods when simultaneous experiments were run under the same conditions, although the standard error for the Barcroft-Dixon method was significantly greater than that obtained with the Winkler procedure. [Variance ratio $S_1^2/S_2^2 = 1.9421$; $F_{0.975}(30, 40) = 1.94$. Fisher-Behren's t test for difference between the means, t not significant at the 5 per cent level.] Manometer experiments were usually of three to four hours duration after an equilibration period of about 15 minutes. Several readings were made during the course of a run. Total change was converted to volume of dry gas at N.T.P. Water bottle experiments were usually run for eight hours. About fifty animals of a given sex and stage of development were used in each experimental bottle or respirometer flask. At the completion of all experiments, the animals were counted and the length of the cephalothorax measured. At the same time a portion of the original tow was preserved for length and weight analysis as described by Conover (1956). A summary of experiments is contained in Table 1.

RELATIONSHIP BETWEEN COPEPOD RESPIRATORY RATES AND BODY MEASUREMENTS

According to Bertalanffy (1951) the relationship between respiration and body size in poikilotherms falls into one of three types. In the first group of organisms metabolic rate is proportional to surface area or $\frac{2}{3}$ power of weight. Mathematically this relationship is expressed as

$$R = bW^{2/3} \quad (1)$$

where R stands for metabolic rate, W for weight and b is a constant of proportionality. In terms of logarithms, expression (1) is written

$$\log R = \log b + 0.667 \log W \quad (2)$$

³ *Pseudocalanus minutus* has been considered synonymous with *P. elongatus* by many authors. Recently Wiborg (1954) has distinguished two species, *Pseudocalanus minutus* (Krøyer) the off-shore form, and *P. elongatus* (Boeck) characteristic of coastal waters. The animals used in these experiments may have been the *elongatus* form but they were not examined in detail.

TABLE 1. Summary of experimental data on the respiratory rates of copepods (adult females)

Date	Number replications	Average length cephalo- thorax (mm)	Average dry weight (mg/1000 cop.)	O ₂ consumed	
				(μl/1000 cop. & hr)	(μl/mg dry wt & hr)
<i>Acartia clausi</i> (L.I.S.)					
July 22 '53	2	0.706	3.80	38.1	10.02
Nov. 19 '53	4	0.770	4.86	28.0	5.77
Dec. 3 '53	1	0.834	5.69	39.6	6.97
Jan. 4 '54	2	0.800	5.23	50.2	9.59
Jan. 26 '54	3	0.816	7.27	54.0	7.45
Feb. 1 '54	3	0.830	7.24	49.6	6.84
July 3 '54	3	0.739	4.19	28.4	6.79
July 10 '54	2	0.723	4.72	29.4	6.23

A. clausi (Southampton Water)

Nov. 11 '54	2	0.824	2.65	28.4	8.78
Nov. 24 '54	2	0.795	3.47	42.6	10.13
Dec. 1 '54	12	0.818	3.57	33.0	9.99
May 9 '55	4	0.961	6.92	84.6	12.19
May 19 '55	3	0.976	8.47	87.5	10.33
May 29 '55*	1	0.958	7.32	92.4	12.66
June 9 '55	2	0.964	8.62	59.9	6.95

A. tonsa (L.I.S.)

July 22 '53	1	0.966	7.52	90.1	11.98
July 29 '53	4	0.931	6.15	81.4	13.23
Aug. 5 '53	4	0.924	5.02	51.5	10.26
Aug. 19 '53	2	0.926	4.67	47.0	10.07
Sept. 23 '53	4	0.945	10.17	76.8	7.55
Oct. 28 '53	4	1.035	7.09	63.2	8.91
Nov. 5 '53	4	1.038	6.71	71.3	10.63
Nov. 19 '53	2	1.053	8.12	69.5	8.56
Dec. 3 '53	2	1.039	7.64	71.8	9.40
Jan. 4 '54	1	0.984	7.14	87.1	12.19
July 3 '54	1	0.976	9.10	87.5	9.62
July 10 '54	2	1.022	10.11	107.3	10.61

A. discandata (Southampton Water)

Nov. 11 '54	2	0.738	1.76	32.8	18.16
Nov. 24 '54	4	0.721	2.34	35.8	15.27
Jan. 13 '55	4	0.742	3.14	31.1	9.88
Jan. 23 '55	10	0.765	3.73	36.7	9.84
Jan. 27 '55	6	0.759	4.99	43.2	8.64
Feb. 10 '55	4	0.767	3.56	39.7	11.14
Feb. 24 '55	12	0.779	4.77	46.6	9.78
Mar. 1 '55	5	0.771	4.80	61.6	12.82
Mar. 8 '55	2	0.786	3.92	65.6	16.72

A. biflosa (Southampton Water)

Feb. 10 '55	2	0.817	4.14	62.2	15.06
Mar. 1 '55	5	0.863	5.66	81.6	14.43
Mar. 8 '55	2	0.876	7.30	96.8	13.29
May 9 '55	2	0.874	7.03	115.6	16.41

TABLE 1. Continued

Date	Number replications	Average length cephalo- thorax (mm)	Average dry weight (mg/1000 cop.)	O ₂ consumed	
				(μl/1000 cop. & hr)	(μl/mg dry wt & hr)
<i>Pseudocalanus minutus</i> (Southampton Water)					
Jan. 23 '55	4	0.799	5.34	56.8	10.61
Jan. 27 '55	6	0.802	6.06	64.8	10.69
Feb. 10 '55	4	0.797	5.53	52.6	9.51
Mar. 8 '55	1	0.914	9.40	76.9	8.15

Temora longicornis (Southampton Water)

Feb. 24 '55	1	0.804	10.34	113.7	11.03
Mar. 8 '55	6	0.942	11.43	148.0	12.87
May 9 '55	2	0.903	12.80	175.0	13.65
May 19 '55	2	0.861	14.28	130.2	9.10
May 26 '55*	2	1.082	14.00	182.6	13.04
June 9 '55	2	0.807	12.54	98.7	7.90

Centropages hamatus (Southampton Water)

May 9 '55	2	1.021	11.72	125.0	10.62
June 9 '55	1	1.005	15.36	91.2	5.93

* Experiment run at Millport, Scotland.

Hence a log plot of respiration against length would give a straight line with slope of 0.667. If it is assumed that the weight or volume of an organism varies as the cube of its length L , Equation (1) becomes

$$R = bL^2 \quad (3)$$

and Equation (2) can be rewritten

$$\log R = \log b + 2 \log L. \quad (4)$$

For the second metabolic type, respiration has a linear proportionality with respect to weight, and thus the slope of a log log plot is 1. There is still a third group of animals in which respiration is related to some power of weight intermediate between 0.667 and 1.

For the Crustacea, it has been shown that the surface rule (type 1) applies to the brine shrimp, *Artemia salina* (Bertalanffy and Krywienczyk 1953) and certain isopods (Bertalanffy and Müller 1943). Raymont and Gauld (1951) found respiration related to body length by a power of 2.19 for several species of copepods and concluded that their metabolism was probably related to surface area. No example of linear proportionality has yet been reported among

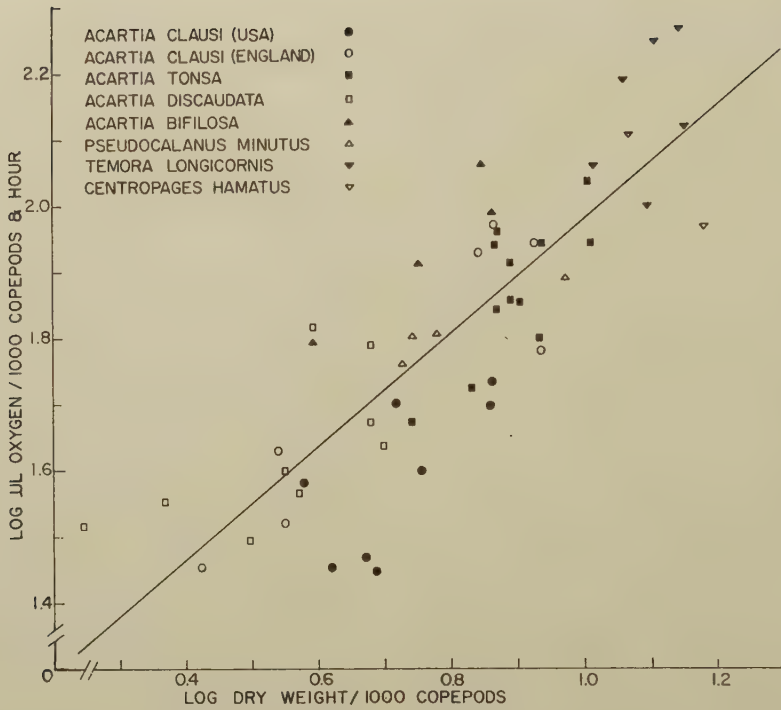


FIG. 1. Log respiration in μL oxygen/1000 copepods and hour plotted against log dry weight in mg/1000 copepods.

the Crustacea, but Bertalanffy (1951) cites several cases among the insects with this metabolic type. The third type has been reported for the isopod *Porcellio* (Will 1952), but the best documented example falling in this category is the kelp crab *Pugettia producta*, studied by Weymouth *et al.* (1944), in which respiration was found to be nearly proportional to the $3/4$ power of weight. In this instance, Equation (1) could be rewritten

$$R = b'W^{3/4} \quad (5)$$

and Equation (3) would become

$$R = b'L^{9/4}. \quad (6)$$

Then, in terms of logarithms,

$$\log R = \log b' + 2.25 \log L. \quad (7)$$

Thus, the regression coefficient 2.19 for copepods [or 2.30 when a single aberrant value was removed (see Raymont and Gauld 1951)] is remarkably close to that for the kelp crab when both are expressed as respiration against length.

In the present paper, the log respiration rate of seven different species of marine copepods has been plotted against both log dry weight (Fig. 1) and log length (Fig. 2). The regression equation for log respiration against log dry weight is $Y = 0.856 X + 1.120$ and that against log length is $Y = 2.713 X - 6.166$. It can be seen that the regression coefficients differ appreciably from the theoretical if either the surface rule (see Equations 2 and 4) or the $3/4$ power rule of Weymouth *et al.* (1944) (see Equation 7) are considered. It would also appear that weight and length are related by an exponent somewhat greater than three ($0.856 \times 3 = 2.568$ not 2.713). Although there is some scatter among the individual observations, the correlation coefficients $r = 0.831$ for log respiration against log weight and $r = 0.674$ for log respiration against log length are both highly significant.

It is recognized that these data extend over a very limited range of size, the largest animals being no more than 57% greater in length than the smallest. In the case of

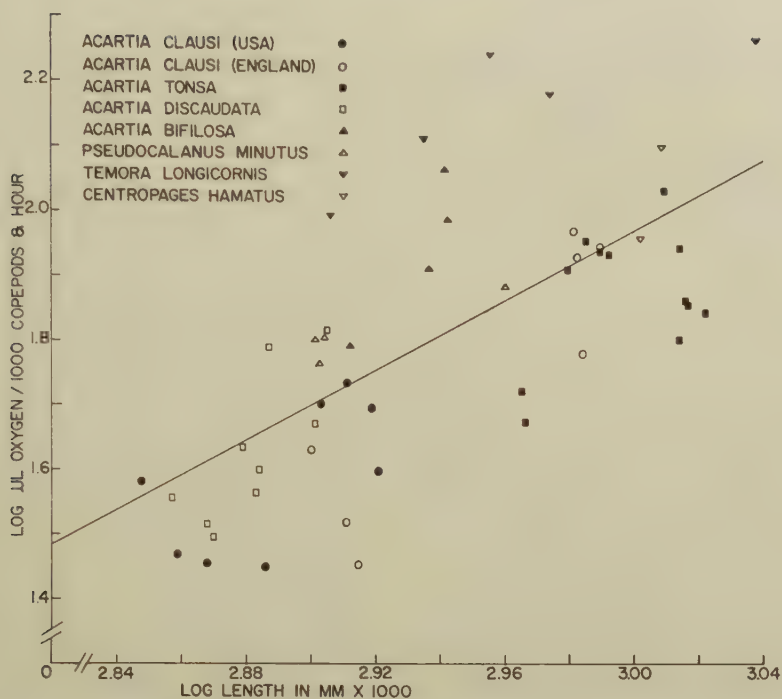


FIG. 2. Log respiration in μl oxygen/1000 copepods and hour plotted against log length in $\text{mm} \times 1000$.

weight, the range is appreciably greater but is still less than an order of magnitude. In studies designed to demonstrate the relationship between metabolism and size, a range of size variation extending over several orders of magnitude is usually employed.

Regression coefficients for individual species are compared in Table 2. Since more observations are available for some species than others, the individual contribution by each species to the plotted regression varies considerably, and the individual regression coefficients do not have the same significance. Yet the variability among regression coefficients particularly for log respiration against log length would suggest that perhaps other factors beside mere size or amount of protoplasm are contributing to the observed respiration. It should be pointed out also that no generally accepted theoretical relationship exists between length and weight (see also Table 1).

It is clear that the relationship between size and metabolism does not follow the surface rule in the instance of the present data. These regression coefficients lie within the

TABLE 2. Regression coefficients for copepods

Log respiration against log dry weight	
<i>Acartia clausi</i> (L.I.S.)	0.819
<i>Acartia clausi</i> (Southampton Water)	0.906
<i>Acartia tonsa</i> (L.I.S.)	1.064
<i>Acartia discaudata</i> (Southampton Water)	0.479
<i>Acartia bifilosa</i> (Southampton Water)	0.955
<i>Temora longicornis</i> (Southampton Water)	0.765
<i>Pseudocalanus minutus</i> (Southampton Water)	0.433
Log respiration against log length	
<i>Acartia clausi</i> (L.I.S.)	2.739
<i>Acartia clausi</i> (Southampton Water)	4.703
<i>Acartia tonsa</i> (L.I.S.)	1.930
<i>Acartia discaudata</i> (Southampton Water)	7.138
<i>Acartia bifilosa</i> (Southampton Water)	7.360
<i>Temora longicornis</i> (Southampton Water)	1.819
<i>Pseudocalanus minutus</i> (Southampton Water)	1.645

range of Bertalanffy's third type of metabolic relationship though they are somewhat greater than that expected if the $\frac{3}{4}$ power rule of Weymouth *et al.* (1944) applied.

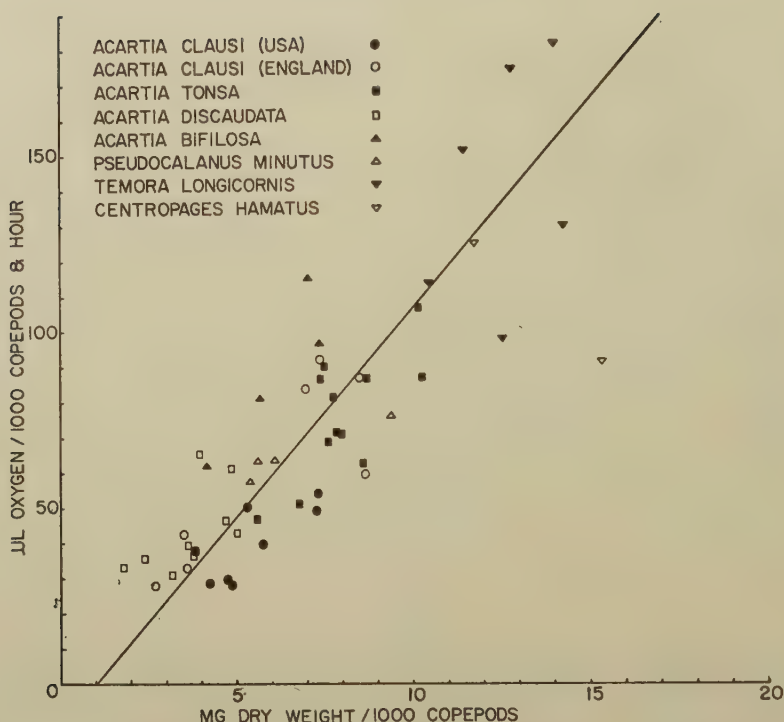


FIG. 3. Respiration in μL oxygen/1000 copepods and hour plotted against mg dry weight/1000 copepods.

In the handling of oceanographic information it is troublesome to apply logarithmic transformation and fractional exponents to large quantities of data. Therefore, it would be desirable to demonstrate that a direct linear relationship between metabolism and body weight, such as would occur if these organisms fitted into Bertalanffy's metabolic type II, was approximately correct at least for the size range of common plankton Crustacea. Such a regression is shown in Figure 3. In this instance the regression equation for respiration against dry weight without benefit of logarithmic transformation is $Y = 11.79X - 10.99$. This equation has a correlation coefficient $r = 0.978$, which is clearly strong evidence that metabolism and body mass within the size range of these neritic copepods is adequately described by direct linear relation. With this mathematical assurance, dry weight has been used in the following sections to provide a direct respiration correction when comparison of metabolism for different sized organisms is desired.

COMPARISON OF RESPIRATION FOR *Acartia clausi* IN LONG ISLAND SOUND AND SOUTHAMPTON WATER

It has already been pointed out that *Acartia clausi* from British waters appeared to have a higher respiration rate than those from L.I.S. However, British *A. clausi* are on the average longer than those from L.I.S. The average length of adult females from L.I.S. for eight collection dates from November to July was 0.778 mm, while that for comparable British animals collected during the same months but a year later was 0.895. The question arises whether the differences in respiration between animals from these two areas is entirely due to size differences.

With this end in view, the respiratory rates for both British and American *clausi* were expressed as oxygen consumed per mg dry weight, and analysis of variance was used to compare the two populations. The results are expressed in Table 3. Inasmuch as the null hypothesis was rejected, it ap-

TABLE 3. Analysis of respiratory data for *Acartia clausi* from Southampton Water and L.I.S.
Null hypothesis: That there is no difference between respiratory rates of *A. clausi* in Southampton Water and L.I.S.

Source of variation	Sum of squares	d.f.	Mean square
Total	65.3110	14	4.6651
Between British and American <i>clausi</i>	23.6678	1	23.6678 = A
Within both groups (pooled error)	41.6432	13	3.2033 = S^2
Within American <i>clausi</i>	18.9110	7	2.7016 = S_1^2
Within British <i>clausi</i>	22.7322	6	3.7887 = S_2^2

Variance ratio $F = S_2^2/S_1^2 = 1.4034$; $F_{0.80}(6, 7) = 1.96$

Since $F < 1.96$, S^2 may be taken as an estimate of a single σ^2 common to both groups.

Variance ratio $F = A/S^2 = 7.3885$; $F_{0.975}(1, 13) = 6.41$

Since $F > 6.41$, reject null hypothesis.

pears highly probable that the respiratory rates of *A. clausi* are different in the two localities at least over the period studied.

In the process of comparing the effect of temperature on the respiration of several species of copepods, Gauld and Raymont (1953) assumed that metabolism is a function of surface area which in turn is related to length by the power 2. They multiplied each respiratory value by the square of the mean length of all experiments over the square of the length for the particular experiment in order to correct for differences in size. If this correction is applied to the present data and the analysis of variance used again to compare populations, it is not possible to reject the null hypothesis that no difference exists. However, the F ratio for within group variance (S_1^2/S_2^2) borders on significance at the 5% level ($F = 3.4700$; $F_{0.95}(6, 7) = 3.87$), which implies that the samples may have been drawn from populations with different values of the population parameter σ .

The difficulty would seem to be associated with the marked differences in respiratory rate between *A. clausi* taken from Southampton Water in the fall of 1954 and those taken the following spring. Although difference in length between fall and spring populations was only slightly more than 0.1 mm, the increase in weight was nearly three-fold (see Table 1). It has already been shown that the metabolism was better correlated with weight than length. With this particular species the correlation between length and respiration did not adequately explain the much greater springtime respiratory rates, so that the error variance was

considerably greater than that obtained from L.I.S. where seasonal respiratory changes were less striking.

SEASONAL DIFFERENCES IN COPEPOD RESPIRATION

It has already been shown that *Acartia clausi* may have a different respiratory rate in L.I.S. than in an estuary on the other side of the Atlantic Ocean, but the lack of a continuous series of observations for this species necessarily limits comparison of seasonal changes.

The best continuous records of seasonal respiratory changes are those for *Acartia tonsa* from L.I.S. and *A. discaudata* from Southampton Water shown in Figure 4. In each case, the measurements extend over the largest portion of the period of yearly occurrence and thus include several generations. Both species had an initially high respiratory rate, falling quite sharply to about half the original figure over a period of several months. In the case of *Acartia tonsa*, there were additional peaks in November and January. By mid-winter when *A. tonsa* had become too scarce for experimental work, its respiratory rate appeared to be falling. In contrast, *A. discaudata* showed an increasing respiration rate toward the end of winter. Although this species did not disappear completely, it became much less important in late winter and spring (Conover 1957).

Available data for *Acartia biflosa*, *Temora longicornis*, and *Pseudocalanus minutus* are found in Figure 5. *A. biflosa* had a decreasing respiratory rate in mid-winter and an apparently increasing rate in the spring.

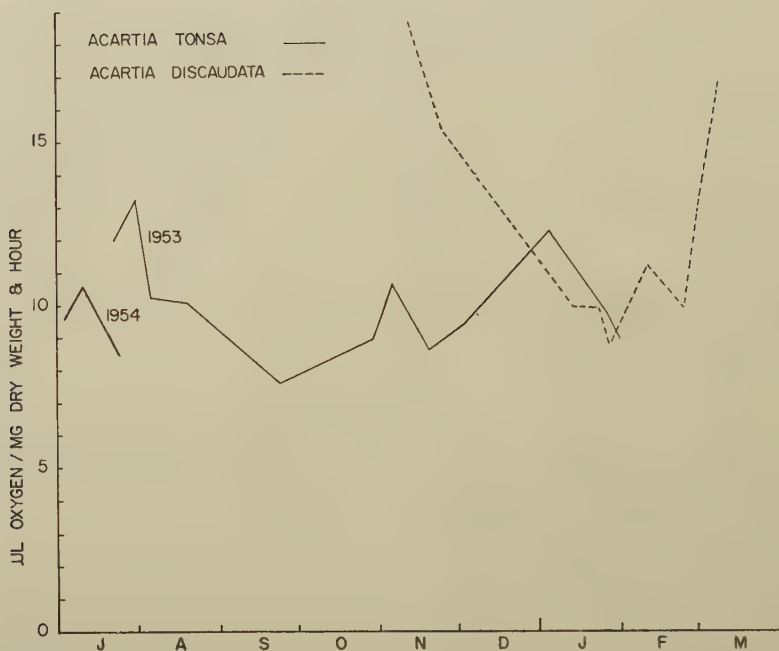


FIG. 4. Respiration at 20°C for *Acartia tonsa* and *A. discaudata* in μl oxygen/mg dry weight and hour measured at intervals over the period from July through March. *Acartia tonsa* from L.I.S. was studied in 1953–1954. *Acartia discaudata* from Southampton Water was studied in 1954–1955.

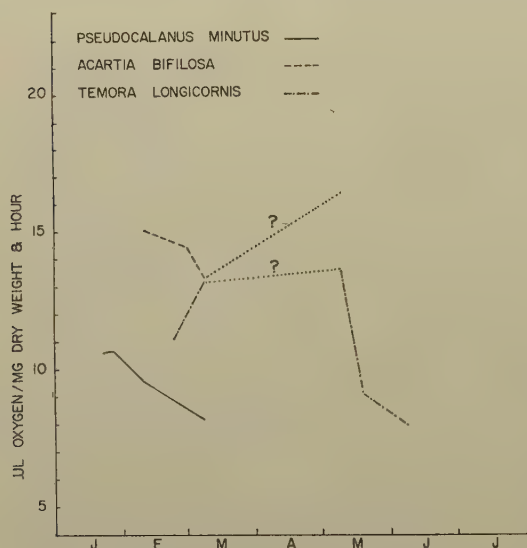


FIG. 5. Respiration at 20°C for *Pseudocalanus minutus*, *Acartia bifilosa*, and *Temora longicornis* from Southampton Water in μl oxygen/mg dry weight and hour measured at intervals over the period from January through June 1955.

Temora longicornis had increasing respiration in March and a declining rate in May. This species is also abundant in summer off Plymouth (Digby 1950), so that more

measurements are clearly desirable. The few measurements for *P. minutus* suggested a generally declining rate, but this species was found in summer plankton off Plymouth (Digby 1950) and should be studied in more detail.

DISCUSSION

Among the poikilotherms two types of temperature compensation have been demonstrated. A single species in a given environment may show acclimatization to seasonal changes in habitat temperature, or the same or closely related species may adjust their metabolism to the prevailing habitat conditions at different latitudes. However, when two populations with different metabolic rates are almost completely isolated from each other as is the case with the populations of the neritic copepod *Acartia clausi* studied here, it can not be stated with assurance whether the differences are the result of physiological adaptation or natural selection for favorable gene combinations. To be sure, Southampton *A. clausi* were taken some 10° of latitude farther north than those from L.I.S. and thus might

be cold adapted but for the fact that the climate of the British Isles is more moderate than that of the Long Island Sound area. Although the mean temperature is virtually the same for each environment (11.6°C for L.I.S., 1953-1954; 11.5°C for Southampton Water, 1954-1955), the summer maximum is higher and the winter minimum lower in the American waters. The experimental temperature 20°C is perhaps higher than British *A. clausi* would encounter in their environment, and they may have developed a degree of stenothermy under the less extreme conditions. In contrast, the L.I.S. *Acartia clausi* used for winter measurements, which came from water colder than 5°C, had a respiratory rate scarcely higher than animals captured in July. Apparently the American *A. clausi* have a greater capacity for control of their metabolism under varying conditions of environmental temperature.

Seasonal adaptation to temperature change would seem inadequate to explain all the variation in respiratory rates observed for animals taken from one environment only. In L.I.S. *Acartia tonsa* had a high rate at opposite extremes of environmental temperature, while in the intervening months the respiration averaged lower. *Acartia discaudata* had a high rate in the fall when the water temperature was about 11°C, a low rate in mid-winter at 5-7°, but a high rate again during a very cold March when the temperature fell to 3°. The respiration of *Pseudocalanus minutus* and *Acartia bifilosa* decreased during February and March 1955, but that of *Temora longicornis* increased over the same period.

At least one other factor might have some influence over the respiratory rate of zooplankton animals. An abrupt rise in the respiration of *Calanus finmarchicus* (Marshall and Orr 1958) and several smaller neritic copepods (Marshall and Orr, personal communication) has been observed in the Clyde at approximately the same time as the spring diatom increase in late April and early May. In laboratory experiments Conover (1956) showed that the respiratory rate of *Acartia* species remained higher when the animals were fed than when they were starved. Although the lack of food does

not seem to have a depressing effect on the respiratory rate of *Calanus* (Marshall and Orr 1958), it remains possible that the metabolism of copepods is in some way geared to their food supply. The advantage afforded the animals by such an arrangement in periods of phytoplankton dearth would be appreciable. On the other hand, there was no significant burst of phytoplankton in L.I.S. when the maximum respiratory rate for *Acartia tonsa* occurred, and it was postulated in an earlier paper (Conover 1956) that the reproductive activity of the animals might account for their higher metabolism. Yet it is clear in the case of *A. tonsa* that there must have been enough food for reproduction to take place. Doubtless sufficient food of the right type is required to stimulate breeding, so that qualitative differences in the phytoplankton which would not show up in purely quantitative studies might be responsible for some of the observed variations in metabolism.

The lack of data from Southampton Water on phytoplankton abundance coupled with limited respiratory measurements makes the interpretation of seasonal variation even more difficult for the other species studied, but if the relative numbers of a given organism are an indication of reproductive success and hence the available food, some rough comparisons can be made using the information available on zooplankton distribution (Conover 1957). *Acartia discaudata* had a high rate during its period of dominance in November, but the rate fell away markedly in mid-winter, even though the copepod continued to be important. In early March the rate increased again, which might be related to a spring augmentation of the food supply. In Southampton Water three species *Acartia clausi*, *A. bifilosa*, and *Temora longicornis* had respiration maxima for the period studied on May 9. All were abundant at the time and may have profited recently from a spring phytoplankton pulse. The respiratory curve for *Temora longicornis* in particular suggests behavior very similar to that observed by Marshall and Orr (1958) for *Calanus*. The few measurements for *Pseudocalanus minutus* indicated a generally declining metabolism through the winter months. This species became important in

Southampton Water in January, and the curve of its abundance paralleled its respiration curve moderately well.

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Aggregates of Bacteria in Sea Water as Determined By Treatment With Surface Active Agents¹

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ABSTRACT

Sea water samples were treated with the surface active agents, Tween 80, MYRJ-52, Renex 31, and Dow Corning Antifoam AF Emulsion to determine their effect on the dispersal of microbes in aggregates. All of these substances in the concentration of 10 ppm increased the plate counts compared to untreated controls. In the case of Tween 80, the most carefully studied of the wetting agents, the results from different water samples were somewhat variable, with an average of almost twice as many colonies developing from the treated sea waters. Mechanical agitation of the sea water had little effect on the number of colonies developing on plates compared to stationary controls. Maximum development of colonies on agar pour plates occurred when sea water samples were treated with 1 to 10 ppm Tween 80. There was evidence for inhibition of pigmented colonies at concentrations of the wetting agent higher than 10 ppm as indicated by serial dilution and replica plate methods.

Direct microscopic observations of microorganisms from water before and after treatment with the surface active agents indicated the occurrence of cells in large clumps, usually on detritus, and individual cells. Less than 13 per cent of the total number of cells was removed from the aggregates by the surface active substances. There was a tendency for the percentage of clumped bacteria to be inversely proportional to the total microscopic count.

The dispersal of bacterial clumps by the surface active agents is attributed to their surface tension depression.

INTRODUCTION

The existence of the microbial flora of aquatic environments in clumps has been suggested by various investigators (Lloyd 1930, Benecke 1933, ZoBell 1946). Such associations may have important ecological implications such as the interchange of metabolites, reduction of exoenzyme escape, and concentration of nutrients. Aggregates of microorganisms complicate cultural methods of enumeration (Butterfield 1933, Jannasch and Jones 1959).

In the present investigation, the effect of surface active agents on the dispersal of aggregated microbial cells in sea water has been studied by colony enumeration and direct microscopic observation. The influence of different concentrations of the surface active agent, Tween 80, on the development of marine microorganisms has been considered.

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METHODS

Sea water samples were collected aseptically, and 99 ml were dispensed into sterile glass containers (6-oz screw-cap prescription bottles or 250-ml Erlenmeyer flasks). Small amounts of surface active agents were added to one set of vessels, while others were left untreated as controls.

The effect of mechanical agitation on the dispersal of aggregates was tested by shaking a group of samples on a gyrotory shaking machine describing a circle about $\frac{3}{4}$ " in diameter.

After treatment, aliquots of the sea water samples were transferred to sterile 100-mm

glass petri plates. Agar pour plates were prepared from one of the following media:

Ingredients	2216E g/L	Glucose g/L	2216E + g (glucose) g/L
Bacto-peptone	5.0	—	5.0
Bacto-yeast extract	1.0	—	1.0
Glucose	—	10.0	10.0
NH ₄ NO ₃	—	1.0	—
K ₂ HPO ₄	—	0.1	—
FePO ₄	0.01	—	0.01
Bacto-agar	15.0	15.0	15.0
Aged sea water, one liter, salinity adjusted to 28 ± 0.5 g/L for all media.			
pH	7.6-7.8	7.6-7.8	7.6-7.8

The replica plate technique of Lederberg and Lederberg (1952) was used for determining the toxicity of Tween 80 on a population of marine bacteria.

Aggregates of bacteria in sea water were observed before and after treatment with surface active agents by the direct microscopic method of Chododny as modified by Jannasch and Jones (1959). This technique involves microscopic enumeration of bacteria on glass slides after concentration by filtration through a membrane filter. Ten microscopic preparations were made of each concentrate, and 25 to 45 fields were counted depending on the amount and distribution of solid particles on the slides.

The surface active agents employed in this investigation were Tween 80, MYRJ-52, and Renex 31 from the Atlas Powder Company, Wilmington, Delaware. Tween 80 is a polyoxyethylene sorbitan monooleate. MYRJ-52 is a polyoxyethylene stearate. Renex 31 is an ether formed by the reaction of ethylene oxide with a highly branched chain synthetic aliphatic alcohol. Renex 31 has a high cloud point which makes it particularly applicable for sea water. All of these surfactants are non-ionic emulsifiers. Dow Corning Antifoam AF Emulsion, a dimethyl polysiloxane fluid containing a few per cent of finely divided silica and conventional emulsifiers, was investigated.

In the tables, per cent error is calculated by $S\bar{x}/\bar{x} \cdot 100$, where $\bar{x}$ is the mean and $S\bar{x}$ is the standard deviation of the mean.

RESULTS

Effect of Tween 80 on plate counts. Sea water samples collected off the Scripps pier were shaken manually for two minutes with and without the surface active agent, Tween 80. The results are summarized in Table 1. The ratio of colonies developing from treated and untreated water varied from 0.95 to 3.2 with an average of 1.8 times more colonies developing on the plates from which the inoculum had been shaken with 10 ppm Tween 80.

Comparison of Tween 80 and AF Emulsion. Sea water samples were obtained off the Scripps pier. They were divided into three groups for treatment expressed as final concentrations: no additions, 10 ppm Tween 80, and 10 ppm AF Emulsion. One set of water samples thus treated was placed on the gyrotory shaking machine for 15 minutes at approximately 260 rpm. The other set remained stationary as a control. In order to determine the effect of 10 ppm Tween 80 on the nutrition of marine microorganisms, a final concentration of 10 ppm Tween 80 was incorporated into one batch of 2216E agar, and the samples were plated in five replicates with 2216E agar and 2216E + Tween 80 agar.

Tween 80 dispersed microbial clumps more efficiently than the AF Emulsion (Table 2). Both surface active substances produced increased plate counts compared to untreated water. The effect is ascribed, mainly, to the influence of the lowered surface tension produced by the surface active materials on microbial aggregates in sea water. The dimethyl polysiloxane polymer is reported to have little effect on the growth of biological materials (personal communica-

TABLE 1. *Effect of 10 ppm Tween 80 on the plate counts of sea water collected off Scripps pier*
Duplicate plates were incubated at 25 ± 1°C for 5 days.

Medium	Number of samples	Ratio of plate counts: treated with Tween 80/no treatment	Per cent error
2216E	6	1.62	13.7
Glucose	6	2.22	13.5
2216E + Glucose	3	1.47	7.4

TABLE 2. *Effect of 10 ppm Tween 80 and 10 ppm AF Emulsion on the plate counts of sea water collected off the Scripps pier*
Five replicates of treatments plated with 2216E and 2216E + 10 ppm Tween media and incubated at $25 \pm 1^\circ\text{C}$ for five days.

	No treatment		AF Emulsion		Tween 80	
	Shaken	Stationary	Shaken	Stationary	Shaken	Stationary
2216E Medium						
Microorganisms/ml	680	630	710	740	1,040	1,330
Per cent error	3.3	12.1	12.0	11.5	13.3	14.1
Ratio—treated/no treatment			1.05	1.17	1.54	2.10
2216E + 10 ppm Tween 80 Medium						
Microorganisms/ml	508	700	750	870	820	1,510
Per cent error	12.3	8.3	7.1	6.7	5.5	10.7
Ratio—treated/no treatment			1.16	1.21	1.47	2.16

TABLE 3. *Effect of various surface active agents on the plate counts of sea water collected off Scripps pier and a local tide pool*
Quadruplicate 2216E plates were incubated at $18 \pm 1^\circ\text{C}$ for 5 and 22 days.

	No treatment		Tween 80 1 ppm		Tween 80 10 ppm		Renex 31 10 ppm		MYRJ-52 10 ppm	
	5 days	22 days	5 days	22 days	5 days	22 days	5 days	22 days	5 days	22 days
Scripps pier water										
Microorganisms/ml	25	71	54	97	56	103	60	105	41	89
Per cent error	12.0	4.2	14.8	9.8	11.6	13.6	7.5	4.3	11.0	5.1
Ratio—treated/no treatment			2.16	1.37	2.24	1.45	2.40	1.48	1.64	1.25
Tide pool water										
Microorganisms/ml	75	107	97	166	113	171	136	200	196	223
Per cent error	8.7	7.0	11.9	11.1	14.6	12.6	6.6	12.8	5.9	6.5
Ratio—treated/no treatment			1.29	1.55	1.51	1.60	1.81	1.87	2.61	2.08

tion from the Dow Corning Corporation), and 10 ppm Tween 80 added to 2216E agar did not change the colony counts significantly in either direction compared to 2216E agar alone.

The surface active agents were no less effective in dispersing bacterial aggregates under stationary conditions than where the sea water samples were agitated. This result indicates that moderate mechanical agitation has little effect on breaking up clumps.

Comparison of Renex 31 and MYRJ-52 with Tween 80. Renex 31 and MYRJ-52 were compared with Tween 80 for their ability to disperse bacterial clumps in sea water. All of these wetting agents were used in concentrations of 10 ppm, and Tween 80 was used in a concentration of 1

ppm as well. Sea water samples were procured off the Scripps pier and from a local tide pool. Each of the experimental samples was shaken on the gyrotory shaking machine for 30 minutes at approximately 260 rpm and plated in quadruplicate. The plates were incubated at $18 \pm 1^\circ\text{C}$ for 5 and 22 days.

In all cases, treatment of sea water with the surface active agents increased the number of colonies developing on agar plates (Table 3). Renex 31 and MYRJ-52 appear to be as effective as Tween 80 in dispersing microbial clumps in sea water. There was little difference in the effectiveness of 1 or 10 ppm Tween 80.

Effect of different concentrations of Tween 80. The problem of the toxicity of the surface active agents and the concentration

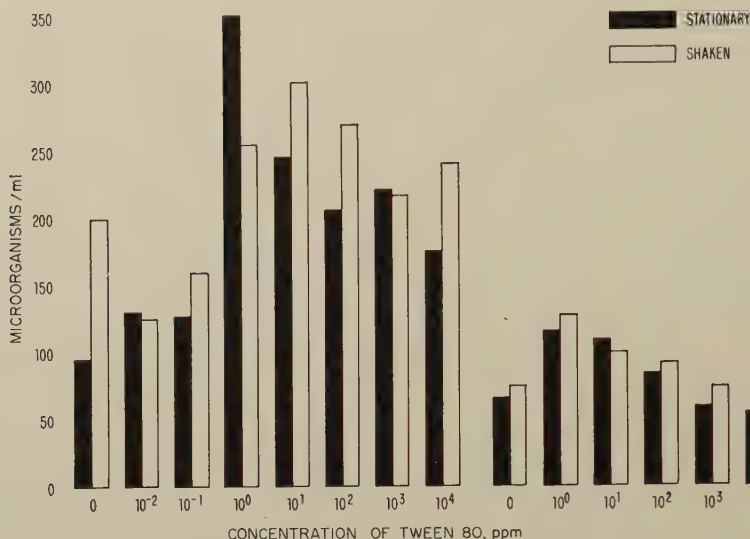


FIG. 1. The effect of different concentrations of Tween 80 in two separate experiments on the number of microbial colonies developing on 2216E agar plates. Quadruplicate plates were incubated at $25 \pm 1^\circ\text{C}$ for five days.

where the substances were most effective for dispersing aggregates was investigated. Surface sea water from off the Scripps pier was treated with concentrations of Tween 80 ranging from 10^{-2} to 10^4 ppm increasing by increments of the power 10. In two experiments one set of 250-ml Erlenmeyer flasks was shaken for 30 minutes at approximately 190 rpm on the gyrotory shaking machine while another set of flasks remained stationary.

The data in Figure 1 indicate that the optimum concentration of Tween 80 for maximum cell dispersion and minimum toxicity is 1 ppm. However, 10 ppm Tween 80 produces a considerably greater drop in surface tension, 4 and 20 dynes per cm depressions for 1 and 10 ppm Tween 80, respectively.

As indicated in Figure 1, there was no consistent difference between the number of colonies developing from water samples that were shaken or stationary. There was a noticeable difference between the types of colonies developing on the agar plates from sea water samples treated with different concentrations of Tween 80. The kinds of colonies progressively decreased in abundance as the concentration of Tween 80 increased as judged by their chromogenic properties, colony characteristics, and cell

morphology. At concentrations of 10^3 and 10^4 ppm Tween 80 almost all of the colonies were colorless, smooth forms. These results suggest that Tween 80 in high concentration is toxic for many marine microorganisms and that resistant forms are greatly dispersed.

In order to obtain more information on the relative toxicity of Tween 80, the development of various heterogeneous populations of marine microorganisms as colonies on 2216E agar plates were tested on the same medium plus Tween 80 in concentration of 10^0 to 10^4 ppm at increments of the power 10. The pattern of microbial growth was transferred from the initial agar plates to the sterile Tween 80 series by the replica plate method. The plates were allowed to set for 24 to 48 hours prior to inoculation to check sterility and remove excess moisture. For each test, five different 2216E agar plates harboring a diversity of aerobic heterotrophic marine colonies were selected and replicated on the Tween 80 plates in descending order of concentration. The number of colonies developing on the various concentrations of Tween 80 is expressed as per cent of untreated control colonies in Table 4. The surface tensions of aged sea water containing Tween 80 were determined with a Du Noüy tensiometer (Table 4).

A progressive toxicity for Tween 80 was

TABLE 4. *Effect of different concentrations of Tween 80 on a population of marine microorganisms determined by the replica plate method of Lederberg and Lederberg (1952)*

Five replicate 2216E plates were incubated at $25 \pm 1^\circ\text{C}$ for 5 days.

Concentration of Tween 80 in ppm	Surface tension (dynes/cm)	Mean % survival of replicated colonies	Per cent error
0	73	100	—
10^0	69	92	1.2
10^1	52	75	1.8
10^2	44	67	1.9
10^3	43	61	1.8
10^4	42	55	2.3

expressed by development of colonies on the plates using the replica plate technique. Where microbial colonies from other water masses were tested by this method, the relative per cent counts were not suppressed to the same extent as those reported in Table 4. However, 10 to 15 per cent inhibition was the minimum observed at the higher concentrations of the surface active agent. A selection for nonchromogenic, mucoid colonies at the higher concentrations of Tween 80 was noticed as before.

Direct microscopic evidence of microbial clumps in sea water. Various kinds of associations between solid material and attached bacterial cells were observed using the Cholodny method. Errors in distinguishing between free and aggregated bacteria may occur if the density of detritus particles is too high in the microscopic preparations. Consequently, the amount of water treated was determined by its content of living and dead particulate matter.

In neritic waters particles were covered or closely surrounded by microorganisms (Fig. 2 and 3). Attachment of bacteria to apparently dead diatoms was observed in the same environment (Fig. 4). Short chains of bacterial cells also are considered as bacterial aggregates (Fig. 5). Clumps such as that shown in Figure 6 were typical for depths of 100 and 200 meters in oceanic areas. Filamentous and twisted forms were observed with apparent spore-forming rods next to amorphous matter. Spirilli-like forms are common, but they have never been observed in clumps (Jannasch and Jones 1959).

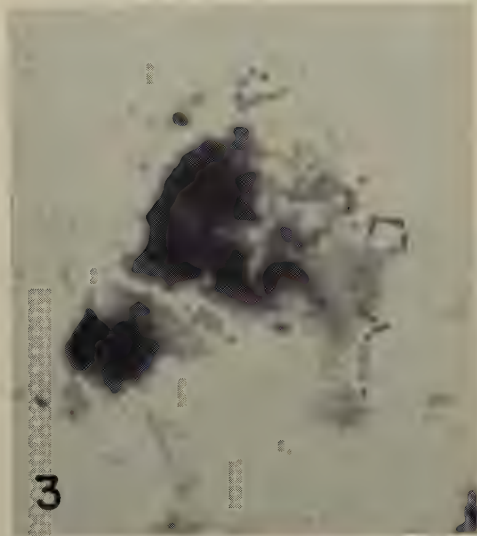
The numbers of bacteria observed microscopically in the two samples reported in Table 3 and five other samples obtained from a vertical cast on the open sea are presented in Table 5. The third and fourth columns of Table 5 present the percentage of bacteria found in aggregates before and after treatment with Tween 80. It was possible to distinguish individual cells even in aggregation as demonstrated in the photomicrographs. The percentage of dispersal of clumps by treatment with Tween 80 was low, particularly in samples containing large numbers of cells. Even so, the estimated total number of microorganisms released from clumps is apparently much larger than the increase of the cell count recorded by the agar pour plate method. In the pier sample (Tables 3 and 5), for example, a 4.4 per cent decrease in the number of clumped organisms resulting from treatment with Tween 80 as determined microscopically corresponded to the dispersal of about 2,500 individual cells compared to a 145 per cent increase in the number of free cells or about 30 colonies by the plate method. Explanations for the considerable differences recorded from the methods have been discussed by Jannasch and Jones (1959).

In contrast to the results obtained microscopically from neritic waters were those of a vertical cast in an oceanic area (Table 5). There was a tendency for the percentage of clumped microorganisms to be lower when

TABLE 5. *Clumped and individual bacteria as observed microscopically before and after treatment with 10 ppm Tween 80*

Location	Total number of bacteria	Per cent clumped bacteria		Drop in percentage of clumped bacteria
		Before treatment	After treatment	
Scripps pier	55,400	26.3	21.9	4.4
Tide pool	46,100	14.7	12.0	2.7
Vertical cast:*				
Surface	24,400	20.2	14.2	6.0
25 m	6,200	41.1	22.5	18.6
50 m	4,300	36.7	23.9	12.2
75 m	7,900	58.4	32.0	26.4
100 m	9,700	29.6	3.8	25.8
200 m	9,100	13.9	6.0	7.9

* At $32^\circ 38' \text{ N}$ latitude; $117^\circ 32' \text{ W}$ longitude.



Microscopic preparations obtained by the Chodny method stained with a 1 per cent solution of erythrosine in 5 per cent phenol, and photographed using a blue filter. FIG. 2. Scripps pier sample, magnification 1,000X. FIG. 3. Tide pool sample, magnification 1,000X. FIG. 4. Scripps pier sample, magnification 750X. FIG. 5. Scripps pier sample, magnification 1,500X. FIG. 6. From 200 meters at 32° 38' N latitude; 117° 32' W longitude, magnification 1,000X.

the total counts were higher. More individual cells were dispersed from aggregates in the samples obtained from depths of 25 to 100 meters than from the surface or from neritic areas. At 200 meters, however, few clumps were observed. There was a high percentage of individual filamentous forms. No comparative values with the plate method are recorded due to the very low plate counts obtained at sea.

DISCUSSION

With the exception of Tween 80, little is known about the effect of the surface active substances on bacterial development. The influence of surface active agents on bacterial metabolism involves charge, structure, and concentration (Hotchkiss 1946, Glassman 1948). Tween 80, in addition to the other surfactants employed, is a non-ionic wetting agent, and there is no way to relate the charge of these compounds to their activity.

Although Ivarson and Pramer (1956) have shown that 25 and 250 ppm Tween 80 had no significant effect on the total number of bacteria obtained by plate counts from a 1/10 dilution of soil, the present results indicate approximately twice as many bacterial colonies obtained by plate counts upon shaking with 1 and 10 ppm Tween 80 in sea water. Explanations for these differences may reside in the lower nutrient concentrations of sea water, which may tend to promote clumping (ZoBell 1946), and the possibility of toxicity from the higher concentrations of Tween 80 employed by Ivarson and Pramer.

The toxicity of Tween 80, especially at high concentrations, was noted during this investigation. Davis and Dubos (1946) showed that small inocula of tubercle bacilli were inhibited by small amounts of unesterified oleic acid present in Tween 80. It is possible that the toxicity of Tween 80 at higher concentrations in these experiments was due to traces of unesterified oleic acid. Tween 80 in concentrations of 1000 ppm has been employed to induce submerged and diffuse growth of the tubercle bacilli (Dubos and Davis 1946). The surface active properties of Tween 80 are considered more important for stimulating submerged growth

of mycobacteria in liquid media than the chemical structure, since nonsurface active oleic acid esters are not effective.

Concentrations of 3,000 to 20,000 ppm Tween 80 caused *Staphylococcus aureus* to grow as a smooth variant in broth but did not affect the colony structure on agar (Gershenfeld and Johnson 1953). Concentrations of 50 and 250 ppm Tween 80 had no inhibitory effect on the development of *Thiobacillus thiooxidans* (Starkey *et al.* 1956). MacLeod *et al.* (1958) tested a variety of surface active compounds for their nutritional effect on a marine *Flavobacterium*. The growth promoting properties of the surface active compounds were attributed to their surface active properties rather than to any capacity to serve either as precursors of cellular lipids or as sources of fatty acids. However, Williams *et al.* (1947) implicated Tween 80 in the metabolism of *Lactobacillus bulgaricus* as a non-toxic source of oleic acid which was essential for growth if biotin was omitted.

In this investigation, the effect of the surface active agents is considered to be primarily physical since the concentrations of the wetting agents were lower than those used by most other investigators. The microbial populations in the sea water were exposed to the surface active substances for only a relatively short period of time before plating with nutrient agar devoid of surfactants. In the experiment where 10 ppm Tween 80 was incorporated into the agar medium, no difference was observed between the colonies on these plates and those containing no Tween 80. However, where the samples were treated with higher concentrations of Tween 80 (10^3 to 10^4 ppm), there was apparent selection in the type of colonies developing. While we are aware of the metabolic effect of many surface active substances, the increase in colony counts resulting from treatment of sea water with the surface active substances in this study is ascribed to the dispersal of portions of microbial aggregates.

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Uptake, Accumulation and Loss of Radioactive Cerium-144 by Marine Planktonic Algae¹

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ABSTRACT

Following the uptake of an element by marine planktonic algae is difficult if the element occurs in both the ionic and particulate states. In this investigation the radioactive cerium (Ce^{144}) occurred mostly in the particulate state when added to sea water. The rate of formation of particles containing Ce^{144} was influenced by dilution, temperature, salinity, added cerium, and the presence of a chelating compound.

A culture of *Nitzschia closterium* containing dividing cells removed all the Ce^{144} originally added to the medium. Different species of algae concentrated cerium from 300 to 3000 times over amounts in the medium in a period of 30 minutes. After 24 hours all 6 species tested had concentrated cerium from about 2,000 to 4,500 times. Radioactive cerium continues to be taken up for as long as three days by non-dividing *Nitzschia* cells kept in the dark. The amount of cerium taken up by *Nitzschia* cells increased as the concentration of cerium in the medium was increased. Particulate cerium became associated with the cells at a faster rate than ionic cerium. The presence of a chelating compound in the medium resulted in a reduction in the uptake of Ce^{144} by *Nitzschia* cells.

Radioactive *Nitzschia* cells resuspended in nonactive medium lost about 25 per cent of their contained Ce^{144} in 5 hours. More Ce^{144} was lost from cells resuspended in 4 portions of nonactive medium, one after the other, than from cells remaining in the first portion of nonactive medium. A larger amount of Ce^{144} was lost from cells to medium containing a chelating compound. The greatest loss of Ce^{144} per unit of protoplasm in cells in nonactive medium was from "biological dilution." This loss amounted to $\frac{1}{2}$ of the contained activity of the cells at each division.

INTRODUCTION

Radioactive fallout from the testing of nuclear weapons indiscriminately contaminates either the oceans, land, or both, depending upon existing meteorological conditions. Up to the present time probably the greatest quantity of fission products added to the oceans has come from weapon tests. Additional fission products in the oceans in the future will originate as wastes from laboratories, power reactors, and plants producing nuclear fuels and materials for nuclear weapons. With the possibility of increased contamination of the oceans with fission products there is a need for determination of the uptake, accumulation, and loss of individual radionuclides by marine organisms. These data can be used to predict the hazards of added radio-

activity to marine populations and the possibility of different radionuclides passing through food webs and occurring in organisms harvested as food for man.

Cerium radionuclides contribute a large portion of the fission-produced materials occurring in sea water soon after the detonation of a bomb. This was shown by a radiochemical analysis on sea water collected near Bikini Atoll in June, 1954, following the hydrogen bomb tests which occurred during the period March to May, 1954 (Miyake and Sugiura 1955). Also, on land the greatest amount of radioactivity from fallout is contributed by the rare earth elements. The average of two complete soil assays, determined two years after the Marshall Islands were contaminated by fallout from the March 1, 1954, nuclear detonation of Operation Castle, gave 84% rare earths, 10% Ru^{106} , 5% Sr^{90} , and less than 1% Cs^{137} (Weiss *et al.* 1956).

The physical state in which an individual fission product occurs when added to sea

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water controls to a great extent its mode of entry and subsequent assimilation by marine organisms. Greendale and Ballou (1954) determined the distribution of fission product elements among the soluble, colloidal, and particulate states by simulating in the laboratory an underwater detonation of an atomic bomb. Although there was some variation in duplicate trials, they found for cerium that approximately 2% was in the ionic state, 4% colloidal, and 94% particulate. Insoluble particles in the oceans can remain in suspension for considerable periods of time, depending upon the turbulence of the water, and can come in contact with surfaces upon which they can be adsorbed or attached (Donaldson *et al.* 1956, and Seymour *et al.* 1957).

In the oceans nonactive cerium occurs in a concentration of only 0.4 μg per liter of water (Goldschmidt 1937). The occurrence of radioactive particles in sea water to which radioactive cerium has been added may be due to the small concentration of nonactive cerium in sea water. This statement is based on a large number of researches which established that radiotracers in minute concentrations (generally 10^{-9} M and below) may exhibit a behavior similar to that of colloids (Schweitzer and Jackson 1952). These substances have been termed radiocolloids. Kurbatov and Pool (1944) have shown that cerium occurs as a radiocolloid.

Species of the filamentous algae, *Oscillatoria* and *Spirogyra*, in a lake contaminated by radioactive wastes concentrated radioactive rare earth elements to the same extent as radioactive phosphorus (Krumholz 1956). Much of this activity from rare earth elements could have been the result of an accumulation of radioactive cerium, since Steel and Gloyna (1955) have shown that freshwater algae in laboratory cultures can remove as much as 95% of the radioactivity contained in an inoculum of Ce^{144} to the culture.

Uptake of Ce^{144} by marine algae may be just as great as that of freshwater algae. This is indicated by findings of the Applied Fisheries Laboratory of the University of Washington (Donaldson 1955) showing that a large fraction of the radioactivity of the

seaweeds, *Caulerpa* and *Halimeda*, collected between March 26, 1954, and June 30, 1955, at Rongelap Atoll, was due to Ce^{144} . Also, planktonic algae take up large amounts of Ce^{144} as shown by radiochemical analysis of plankton collected during USAEC Operation Troll. Actually 80 to 90% of the activity in these samples was due to Ce^{144} and its daughter Pr^{144} .

The purpose of the present investigation was (1) to determine the physical state of Ce^{144} when added as a cerous nitrate solution to sea water, (2) to test the effect of certain factors upon the rate at which particles of Ce^{144} are formed, (3) to measure the accumulation of Ce^{144} by marine planktonic algae, (4) to compare the rates of uptake of ionic and particulate Ce^{144} by algae, and (5) to follow the loss of Ce^{144} from algal cells.

The authors wish to thank Dr. James R. Lance for the *Platymonas* sp., Dr. M. Fudinoga for the *Thalassiosira* sp., and Dr. John H. Ryther for the *Amphidinium klebsi* and *Porphyridium cruentum* cultures.

MATERIALS AND METHODS

The accumulation of radiocerium was measured in the following planktonic algae:

CHLOROPHYCEAE	DINOPHYCEAE
<i>Carteria</i> sp.	<i>Amphidinium klebsi</i>
<i>Platymonas</i> sp.	
BACILLARIEAE	RHODOPHYCEAE
<i>Nitzschia closterium</i>	<i>Porphyridium cruentum</i>
<i>Thalassiosira</i> sp.	

All species were in a high state of purity, having been grown only in inorganic medium, and thus were sufficiently free from bacteria to make their use valid.

The culture medium used in all experiments consisted of natural sea water enriched with Miquel's solutions (Rice 1953). This medium was filtered through a millipore filter prior to using, and algal cultures were grown in Erlenmeyer flasks of a capacity twice the volume of medium used. After the appropriate number of cells and the desired amount of radioactivity had been added to the medium, the cultures were grown in a culture cabinet (Rice 1953) in

constant illumination with daylight fluorescent lamps at a temperature of $22 \pm 2^\circ C$.

The number of cells per liter was determined after vigorously shaking the culture to distribute the cells evenly throughout the medium. The average of two cell counts with an improved Neubauer haemocytometer was used to calculate the population size.

The radionuclide, Ce^{144} , used in this investigation was supplied on authorization from the Isotopes Division, United States Atomic Energy Commission. It has a half-life of 282 days and decays into praseodymium-144, a radioactive daughter product with a half-life of $17\frac{1}{2}$ minutes. It was obtained in the form of cerous nitrate in acid solution.

Radioactivity of samples was measured with an end-window Geiger-Müller tube and a conventional scaler. If separation of Ce^{144} and Pr^{144} had occurred, time was allowed for their return to secular equilibrium before determining the radioactivity in the sample. Appropriate corrections were made for geometry, coincidence, background, and decay. Sample thickness was such that no correction for self absorption was necessary. Results presented are based on duplicate samples. Details of sample preparation will be given as the various experiments are described.

EXPERIMENTAL RESULTS

Most previous investigations of the uptake of inorganic materials from sea water by planktonic algae have been limited to elements present in the ionic state. In this investigation it will be shown that cerium occurs in sea water in both the ionic and particulate states. This makes it difficult to determine the relative importance of the different mechanisms responsible for the observed uptake. No doubt the ionic and particulate components are not equally available for uptake by the cells. Since the Ce^{144} in fallout may be in a different chemical combination from the Ce^{144} used in this investigation, experiments have been included to show that the addition of Ce^{144} as cerous nitrate to sea water results in the formation of radioactive particles. Also,

the rates of formation of these particles will be shown to vary with experimental conditions.

Factors influencing the formation of Ce^{144} particles in sea water

The physical state of Ce^{144} was determined by filtering sea water to which radioactive cerium as cerous nitrate had been added. Any radioactivity on the filter could result from Ce^{144} in the ionic state being adsorbed or from the retention of particles containing Ce^{144} . If only radioactivity from particles is retained on the filter, the rate of formation of particles can be followed easily and accurately. A certain amount of ionic Ce^{144} , theoretically, would be required to completely saturate the filter pad by surface adsorption. Thus there would be no further increase of radioactivity as additional volumes of sea water culture medium are filtered. If adsorption does not contribute significantly to the activity retained on the filter, particles equally distributed throughout the medium would be retained in direct proportion to the volume of medium filtered.

To measure changes in the radioactivity of the filter, volumes of 10, 20, 30, 40, 50, and 60 cc of medium, containing 6.2 microcuries of carrier-free Ce^{144} per liter, were each filtered through a separate millipore

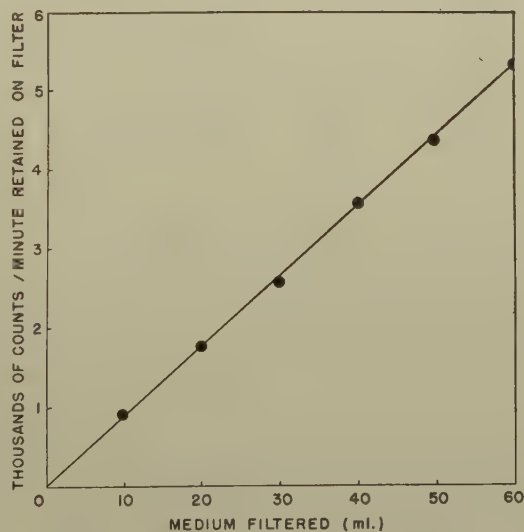


FIG. 1. Radioactive cerium retained on millipore filter from culture medium.

filter. Activity retained on the filters formed a straight line when plotted against the volume of medium filtered (Fig. 1). It can thus be concluded that the addition of Ce^{144} in the form of cerous nitrate to sea water culture medium results in the formation of particles which can be filtered from the medium. The radioactivity of the filter is due to particles of Ce^{144} and not to adsorption of Ce^{144} ions.

A number of factors were tested that might be in operation in the oceans or enter into laboratory experiments to influence the rate of formation of particles of Ce^{144} . These include dilution, temperature, salinity, the concentration of cerium, and the addition of a chelating compound.

Dilution. The effect of dilution was determined by adding 5 microcuries of Ce^{144} to each of 3 different volumes of culture medium (Table 1). The greater the dilution of Ce^{144} , the larger was the per cent of Ce^{144} occurring in the particulate state.

Temperature. There was a faster rate of particle formation at 22.6°C than at 3.1°C (Table 2).

Salinity. Since the quantity of salts present in water may influence the amount of Ce^{144} occurring in the particulate state, sea water was diluted with distilled water to give several salinities (Table 3). At some salinities there appeared to be a slight in-

TABLE 1. *Effect of diluting Ce^{144} in sea water upon the percentage retained as particles on a filter*

Hours	Volume (ml)		
	250	1000	3000
0.5	6.5	21.9	42.8
24.0	15.7	40.2	100.0

TABLE 2. *Percentage of Ce^{144} retained on a filter as particles from medium kept at different temperatures*

Hours	Temperature (centigrade)	
	3.1°	22.6°
2.5	4.0	9.4
5.5	4.7	10.2
10.0	7.3	17.0
24.0	7.0	14.2

TABLE 3. *Percentage of Ce^{144} retained on a filter as particles from medium with different salinities*

Salinity ‰	Hours	
	2	24
36	6.4	9.9
31	7.2	8.1
26	6.6	7.6
20	6.8	11.5
15	7.9	10.6
10	9.4	12.8
5	11.6	16.4
*0	41.1	38.9

* Distilled water

TABLE 4. *Percentage of Ce^{144} retained on a filter as particles from medium containing different amounts of nonactive cerium*

Hours	Added cerium (μg)		
	0	57	900
0.5	5.3	38.6	100.0
24.0	8.2	100.0	100.0
96.0	16.2	100.0	100.0

TABLE 5. *Effect of Versene upon the percentage of Ce^{144} retained on a filter as particles from medium*

Hours	No Versene added	0.1 g Versene added
0.5	31.1	1.5
21.0	37.3	2.2
45.0	36.2	3.8
69.0	34.9	3.0
93.0	33.1	2.9

crease in the amount of Ce^{144} occurring in the particulate state. However, the greatest amount of Ce^{144} occurred as particles in the distilled water, which was also tested.

Concentration of cerium. Adding increased amounts of cerium, as cerous nitrate, to the medium resulted in a greater amount of the Ce^{144} being retained as particles on the filter (Table 4).

Chelation. The addition of a chelating compound such as Versene, which is a sodium salt of ethylene-diamine-tetraacetic acid, to culture medium resulted in a smaller percentage of Ce^{144} being retained on the filter (Table 5).

Importance of "growth" of particles containing Ce^{144} on the rate of removal of Ce^{144} from sea water

The rate at which Ce^{144} occurs in the particulate state may be speeded up by the "growth" of particles formed immediately upon addition of Ce^{144} to the medium. To measure the influence of these particles upon the total amount of Ce^{144} removed by filtering, one of two identically prepared volumes of culture medium was filtered just after adding the Ce^{144} , while the other volume of medium was not filtered. When the filtered medium was again filtered, much less Ce^{144} was retained on the filter, only 1 to 2% occurred as particles. By comparison, an increase of 14% of Ce^{144} from the unfiltered medium was retained as particles. Since the formation of new particles in these two volumes of medium probably occurred at about the same rate, it can be concluded that the "growth" of particles formed immediately upon adding the Ce^{144} to the medium is more responsible for the occurrence of Ce^{144} in the particulate state with time than is the formation of new particles.

Uptake of Ce^{144} by Nitzschia cells

Since the addition of the radioactive Ce^{144} as cerous nitrate to culture medium results in the formation of particles, it was necessary to use a method of removing cells from the medium which would exclude as much Ce^{144} not associated with the cells as possible. The per cent of Ce^{144} in medium that can be removed by centrifuging was determined and compared with that removed by filtering. Also, the loss of Ce^{144} from medium with time due to adsorption on the walls of the culture flasks was measured, since the experiments on the uptake of Ce^{144} by phytoplankton were to be followed for several days.

The culture medium used in this experiment contained 6.8 microcuries of Ce^{144} per liter. Periodically, samples of 5 cc each were removed, evaporated to dryness, and the contained activity determined. Also, samples of 25 cc each were centrifuged, while other duplicate samples were filtered. The centrifuged medium was decanted, and the centrifugate was suspended in nonactive

TABLE 6. Loss of Ce^{144} with time from culture medium in a standing culture and by centrifuging and filtering

That lost by centrifuging and filtering is based on the amount remaining in the standing culture medium at the time of testing.

Days	Percentage Ce^{144} lost from medium		
	Standing culture	Centrifuging	Filtering
0			5.9
7	15.0	1.7	7.7
10	24.0	2.6	8.0
14	31.7	3.1	7.8
18	34.2	8.2	8.5

medium and filtered on a filter pad, so that the activity centrifuged from the medium could be measured.

Radioactivity continued to be lost from the medium to the sides of the flask upon standing (Table 6). For the first 14 days only 2 to 3% of the activity remaining in the medium was lost by centrifuging. After 18 days the amount lost by this method had increased to over 8%. The per cent of activity removed by filtering did not vary much over the entire period of time tested. From the data presented in this table it is obvious that centrifuging is the better method for separating cells from the radioactive medium during the first 14 days. In the remaining experiments Ce^{144} in cells was determined by centrifuging the cells from either 25 or 40 cc of medium, decanting the active medium, resuspending the cells in nonactive medium, filtering the cells on a millipore filter, and measuring the Ce^{144} in the cells.

In cultures not shaken. The uptake of Ce^{144} from culture medium by dividing cells was followed in a culture started with 4×10^7 *Nitzschia* cells and 6.2 microcuries of Ce^{144} per liter. A control culture to which no cells were added was started with the same amount of Ce^{144} . The activity remaining in the medium of the control was determined at the same time the *Nitzschia* culture was sampled.

The curve representing the counts per minute associated with cells (Fig. 2) follows rather closely the curve representing the number of cells present. There was

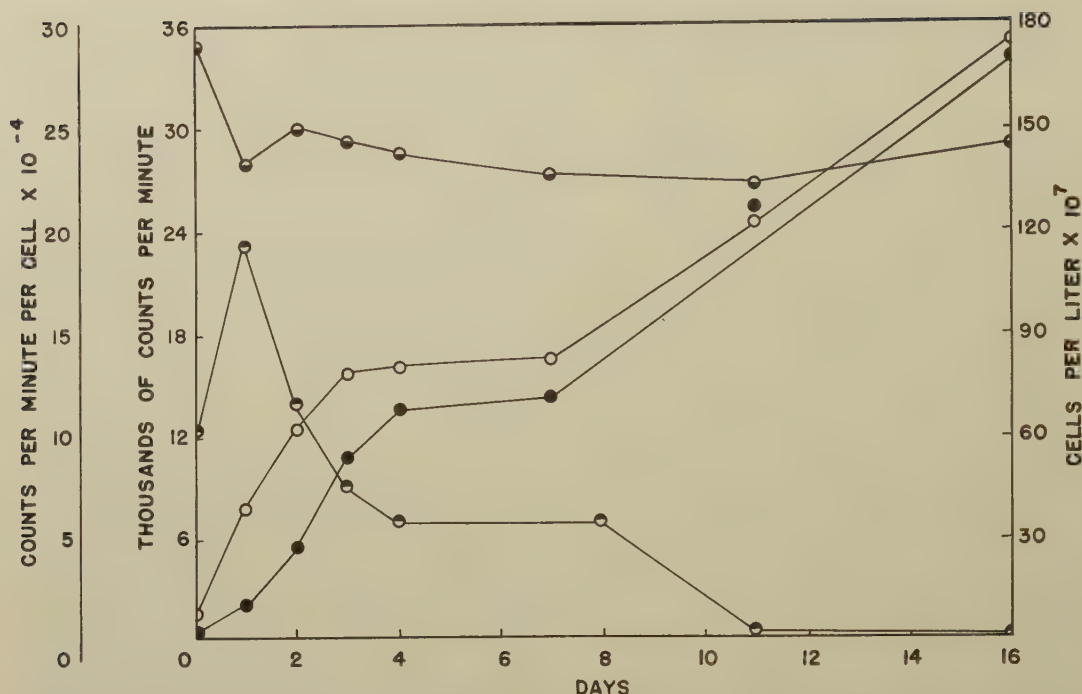


Fig. 2. Uptake of Ce^{144} by dividing *Nitzschia* cells and loss of Ce^{144} from medium of the control.

- Counts per minute contained in 40 cc. of medium from control culture
- Counts per minute in cells from 40 cc. of medium
- Population growth curve of *Nitzschia* cells
- Counts per minute per cell

very little increase in the number of cells or the uptake of activity from the fourth day until after the seventh day. The factor or factors responsible for this are not known. The amount of activity in the cells on the sixteenth day represents almost all that was present in the medium at the beginning of the experiment. This uptake by the cells occurred, even though the activity remaining in 40 cc of medium of the control on this date had decreased from about 34,500 to 29,100 counts per minute. The cells removed the activity at a sufficient rate to prevent much from being adsorbed or attached to the walls of the flask. The greatest amount of activity per cell occurred at the end of 24 hours.

In cultures shaken. Forces that enhance the mixing of sea water in the oceans will also increase the chances of phytoplankton and particulate material coming in contact. This may speed up the association of Ce^{144} particles with phytoplankton cells. To

measure in the laboratory the effect of mixing on the rate of uptake of Ce^{144} from the medium, some cultures were shaken for 30 minutes on an automatic shaker, while others were not. The *Nitzschia* cultures were started with 24×10^7 cells and 5.9 microcuries of Ce^{144} per liter. Controls were also prepared with the same amount of Ce^{144} in the medium, but containing no cells. Some controls were shaken for 30 minutes on the automatic shaker while others were not.

The Ce^{144} in the medium of the control that was shaken decreased 37% more than in medium which was not shaken. This was probably due to more Ce^{144} coming in contact with the sides of the flask and being adsorbed in greater amount in the shaken than in the unshaken control. *Nitzschia* cells in the shaken cultures took up 39% of the activity in the medium, while cells in the unshaken cultures removed 30%. However, since there was less Ce^{144} remaining

in the medium in the cultures that were shaken, the cells in the unshaken cultures contained more Ce^{144} .

Concentration of Ce^{144} by planktonic algae

In the previous experiment considerable Ce^{144} was taken up by the *Nitzschia* cells in only $\frac{1}{2}$ hour, while the greatest amount of activity per cell occurred at 24 hours. The extent to which Ce^{144} was concentrated at these time intervals and under the same experimental conditions by six species of algae was determined. The concentration of activity by the algae was calculated on a wet weight basis following the method described by Rice (1956), except that the cells were centrifuged from the medium. At the end of only $\frac{1}{2}$ hour some of the cells had concentrated Ce^{144} from about 2,000 to 3,000 times over amounts occurring in an equal weight of medium (Table 7). After 24 hours all algae had concentrated the radionuclide from about 2,000 to almost 4,500 times.

In all further experiments *Nitzschia closterium* cells were used, since at the end of 24 hours the *Nitzschia* cells had concentrated Ce^{144} the least of all species. Thus following the uptake of Ce^{144} by *Nitzschia* cells under the various experimental conditions would more nearly approximate the least amount of Ce^{144} accumulated by these species of planktonic algae.

*Uptake of Ce^{144} by various population sizes of *Nitzschia* cells*

Since there is always a standing crop of phytoplankton in nature which can remove part, if not all, of any radionuclide intro-

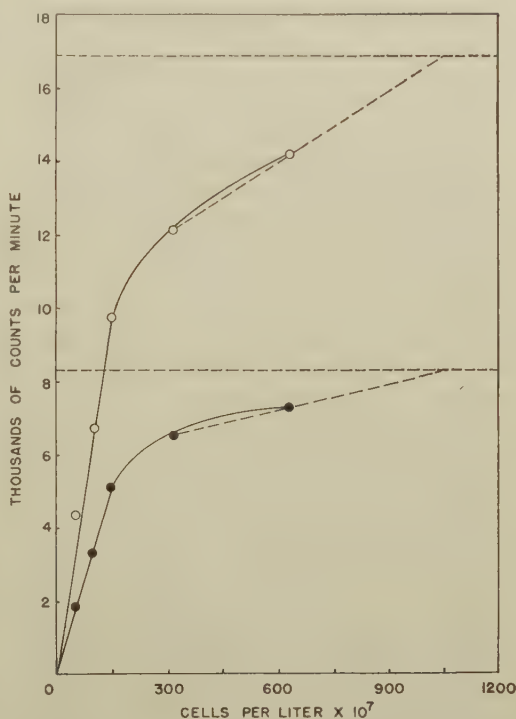


FIG. 3. Uptake of Ce^{144} by different population sizes of *Nitzschia* after 30 minutes in active medium.

- Populations of cells in medium containing 3 microcuries Ce^{144} per liter
- Populations of cells in medium containing 1.5 microcuries Ce^{144} per liter

duced into the oceans, the immediate uptake of Ce^{144} by various population sizes of *Nitzschia closterium* was tested. Population sizes of 48, 83, 157, 313, and 625×10^7 cells per liter were used to start cultures containing 3 microcuries of Ce^{144} per liter. Also another series of cultures was prepared in the same manner, except these cultures contained 1.5 microcuries of Ce^{144} per liter.

The uptake of Ce^{144} in 30 minutes by the different population sizes in cultures containing the two concentrations of activity followed the same pattern, as shown in Figure 3. In cultures containing the largest population size about 85% of the Ce^{144} was removed from the medium by the cells. From these curves it can be concluded that population sizes of a million or less, which ordinarily occur in nature, or even larger populations such as those during blooms, would not immediately remove a very large

TABLE 7. Number of times cerium is concentrated over the amount in sea water by planktonic algae

Algae	Concentration of Ce^{144}	
	0.5 hours	24 hours
<i>Carteria</i> sp.	314	2,422
<i>Platymonas</i> sp.	1,843	2,127
<i>Nitzschia closterium</i>	518	1,970
<i>Thalassiosira</i> sp.	2,610	3,346
<i>Amphidinium klebsi</i>	3,001	4,498
<i>Porphyridium cruentum</i>	802	3,344

amount of the Ce^{144} added to the water. To estimate the smallest population size of *Nitzschia* cells which might conceivably remove all the Ce^{144} from the medium, a straight line was drawn in Figure 3 between the last two points, and extended on to the dotted line representing the activity originally added to the medium from which the cells were removed. The population size required to remove all the Ce^{144} from the medium would actually be larger than this population of about $1,050 \times 10^7$ cells per liter, since the curve tends to level off as the number of cells increases. From this experiment it can be concluded that the immediate uptake of Ce^{144} by a population of phytoplankton which might occur in the oceans would represent a small percentage of that added to the water.

Uptake of Ce^{144} by non-dividing Nitzschia cells

In the oceans under adverse conditions, such as too low a temperature, a shortage of nutrients, or the lack of sufficient light, phytoplankton may not divide or do so very slowly. To measure the removal of Ce^{144} by populations of non-dividing *Nitzschia* cells, cultures were prepared with cells that had been kept in the dark until cell division had ceased. Cultures were then started with either 26, 51, or 102×10^7 cells per liter in medium containing 30 microcuries of Ce^{144} per liter. During the experiment the cultures were retained in the dark to prevent the cells from dividing.

The amount of activity taken up by the cells in $\frac{1}{2}$ hour did not increase in proportion to the number of cells present (Table 8). Activity continued to be removed from the medium by all three population sizes for 72 hours. At this time the uptake of activity

TABLE 8. Percentage uptake of Ce^{144} by non-dividing *Nitzschia* cells at different population sizes and after various exposure times

Population size (10^7 cells/liter)	Hours				
	0.5	24	48	72	144
26	6.1	16.2	21.9	32.4	34.7
51	12.2	24.9	32.4	40.0	47.7
102	16.6	30.2	35.6	49.2	48.6

by the largest population of cells ceased with about 50% of the Ce^{144} having been removed from the medium.

Effect of carrier cerium on the division rate of Nitzschia cells and on the uptake of Ce^{144} by them

It has been shown for many elements that uptake by planktonic algae increases as the concentration of the element in the medium increases, when other factors are kept constant. However, high concentrations of some elements have been found to reduce the division rate of algal cells. Thus the effect of increased concentrations of cerium upon the division rate of *Nitzschia* cells was determined in concentrations of 1, 4, 16, 57, 230, 919, 3,678, and 14,713 μg cerium per liter of medium added as cerous nitrate.

Only the highest concentration of cerium appeared to have any effect on reducing the division rate. All cultures were started with the same number of cells and were grown for 10 days in medium containing the added cerium. The medium with the highest concentration of cerium had a pH of 6.93. It is known that this is an unfavorable pH for the growth of these cells, which

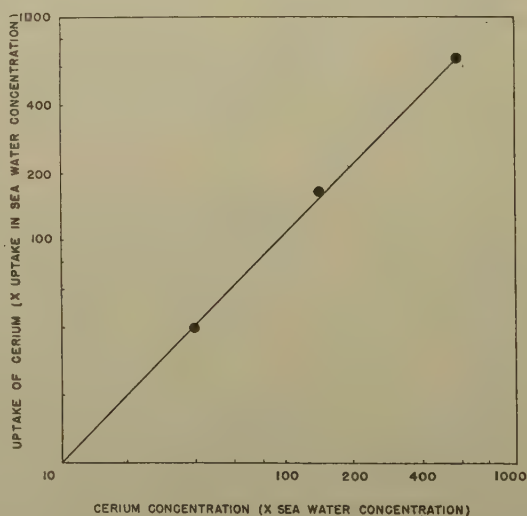


FIG. 4. Uptake of cerium by *Nitzschia* cells grown in medium containing increasing amounts of cerium. (For example, cells grown in medium containing 40 times as much cerium as present in sea water take up 40 times more cerium than when grown in the sea water concentration.)

may partially account for the reduced division rate observed. The next highest concentration of cerium, 3,678 μg per liter, is several thousand times greater than that normally occurring in sea water and probably would never exist in the oceans.

The uptake of cerium by cells in medium with concentrations of cerium equal to 40, 143, and 575 times that occurring in sea water was followed for 7 days. The uptake of cerium increased as the concentration of cerium in the medium increased (Fig. 4). This increase was proportional to the amount of cerium added; for example, if the cerium concentration was increased 40 times over sea water concentrations, cells grown in this medium took up about 40 times more cerium than cells grown in culture medium without added cerium.

*Comparison of the rates of uptake of ionic and particulate Ce^{144} by *Nitzschia* cells*

It has already been pointed out that the cerium radionuclides occur mostly in the particulate state in the oceans. Also, the removal of a part of the Ce^{144} by filtering and centrifuging is evidence that some of it occurs in a particulate state. Actually, it was found that almost all of the Ce^{144} remaining in a four-month-old volume of culture medium could be removed by filtering. Thus, in this medium all the Ce^{144} was considered to be in a particulate state. Uptake

of activity by *Nitzschia* cells from this medium would indicate the rate of uptake of particulate Ce^{144} .

In order to obtain Ce^{144} in the ionic state, 0.5 cc of a solution of Ce^{144} was placed in a semipermeable membrane bag and immersed in a 250-cc volume of culture medium. The medium was stirred constantly, and at intervals 5-cc portions were removed, evaporated to dryness in a plastic Petri dish, and the activity in the sample was measured. There was a rather rapid rate of passage of 12.5% of the Ce^{144} through the membrane bag (Fig. 5).

Upon dialyzing into the culture medium the ionic Ce^{144} might form particles before sufficient amounts could be obtained to follow its uptake by the cells. To reduce the possibility of this occurring, *Nitzschia* cells were placed in the medium into which the semipermeable membrane bag was immersed. Under these conditions it would be possible for the cells, if they possessed the capacity, to take up the ionic Ce^{144} as it passed from the semipermeable membrane bag into the culture medium.

Uptake of Ce^{144} from medium under these conditions and from medium containing all the Ce^{144} in the particulate state was followed from 250-cc volumes of medium containing 12×10^7 *Nitzschia* cells per liter. Both volumes of medium were stirred continuously. In these uptake experiments a new

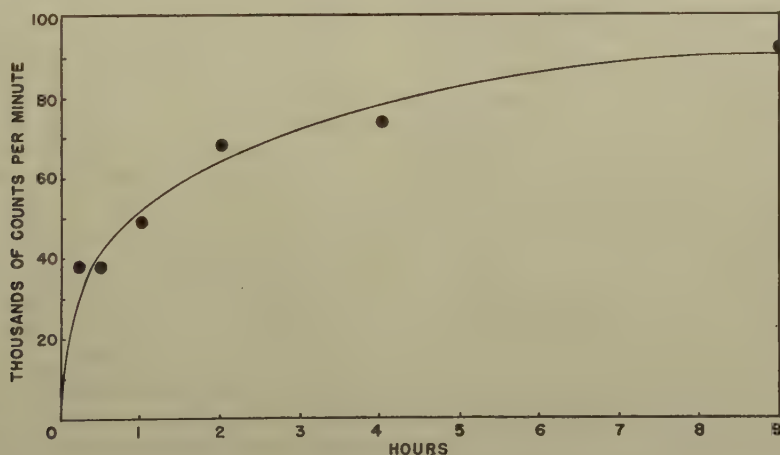


FIG. 5. Dialysis of Ce^{144} into culture medium from a semipermeable membrane bag which contained an initial activity of 740,000 counts per minute.

volume of medium was used for each time interval.

The particulate Ce^{144} was taken up by the cells at a faster rate than the ionic form (Table 9). The one-half hour uptake of ionic Ce^{144} was not determined. Also, the uptake of the cerium radionuclides in the oceans may occur at a very rapid rate, since Greendale and Ballou (1954) found 98% of the cerium radionuclides in the particulate and colloidal states by simulating conditions of an underwater detonation of an atomic bomb.

Uptake by Nitzschia cells of Ce^{144} from medium containing a chelating substance

In a previously discussed experiment Versene was found to reduce the amount of Ce^{144} occurring as particles in the medium. Since it has already been observed that particles are taken up faster than the ionic material, it was of interest to follow the effect of Versene on the uptake of Ce^{144} by *Nitzschia* cells. Cultures with 4×10^7 cells and 8 microcuries of Ce^{144} per liter were prepared. One culture was retained as a control and no Versene was added to it. To one culture 0.025 gram of Versene per liter was added, to another culture 0.05 gram per liter, and to a third culture 0.1 gram per liter of Versene was added.

The uptake of Ce^{144} by *Nitzschia* cells in all three cultures containing Versene was less for the first three days than that taken up by cells in the control culture (Table 10). After 93 hours the total amount of Ce^{144} taken up by the cells in the control and in the cultures with the two smallest concentrations of Versene was not significantly different. However, less activity was taken up by the cells in the culture containing the largest concentration of Versene than in any of the other cultures.

Loss of Ce^{144} from Nitzschia cells

When only a single addition of fission

TABLE 10. *Effect of Versene in the medium on the percentage uptake of Ce^{144} by Nitzschia cells*

Hours	No Versene added	Versene added		
		0.025 g/L	0.05 g/L	0.1 g/L
0.5	7.8	0.3	0.1	0.1
21.0	29.8	1.3	0.8	4.8
45.0	37.0	8.9	5.4	1.8
69.0	32.5	27.0	22.7	8.3
93.0	31.3	32.4	33.4	25.5

products is made to the ocean or when long intervals of time occur between additions, phytoplankton might not be important in passing Ce^{144} to higher trophic levels. This would be true only if the cells could not retain most of the activity taken up. Even though Ce^{144} is concentrated many times over amounts in sea water and may not be lost again to the sea water, it is still possible for the cells through their own division to dilute the activity in their protoplasm. This reduction of activity in the protoplasm by division of cells in water containing no activity is referred to as "biological dilution."

Dividing cells resuspended in nonactive culture medium. The loss of Ce^{144} to the medium from dividing *Nitzschia* cells was found to be small. Cells were first grown for 10 days in culture medium containing 6 microcuries of Ce^{144} per liter. They were then centrifuged from the active medium and resuspended in nonactive medium. This culture was divided into ten 100-cc cultures and placed in the culture cabinet where cell division would continue. The activity in both the cells and medium was measured periodically over a period of 42 days, and the percentage of activity in the medium was calculated.

The greatest amount of activity occurred in the medium at the time the first sample was taken (Table 11) and continued to decrease for the next seven days. After this time there was not much change in the amount of activity occurring in the medium. While the cells immediately lost about 25% of their original activity to the medium, at 42 days they had lost about 80%, mostly by "biological dilution." During the 42 days the cell population increased from 36×10^7 to 205×10^7 cells per liter, or there was a

TABLE 9. *Percentage uptake of ionic and particulate Ce^{144} by Nitzschia cells with time*

Hours	Ionic	Particulate
0.5		52.4
9.0	36.3	83.0
24.0	56.9	84.6

TABLE 11. *Loss of Ce^{144} from active cells to the medium*

Days	Population size (10 ⁷ /cells/liter)	Percentage Ce^{144} in medium
0.2	36	25.2
1.0	35	17.9
4.0	68	12.7
7.0	85	7.2
14.0	91	6.5
21.0	94	9.4
28.0	172	8.2
36.0	180	3.4
42.0	205	6.4

TABLE 12. *Loss of Ce^{144} from cells to medium in repeated washings*

Washings	Percentage Ce^{144} lost to medium
1	7.7
2	8.1
3	7.2
4	8.2

TABLE 13. *Increased loss of Ce^{144} from *Nitzschia* cells as a result of adding Versene to the medium*

Hours	Percentage
1	3.9
4	16.8
24	31.1
48	21.6
96	19.1

total of 2.42 divisions. With a 50% reduction in activity in a cell at each division, after this number of divisions a cell would contain only about 18% of its original activity.

Cells centrifuged and resuspended in sequence in four different volumes of medium. Radioactive phytoplankton floating in non-active sea water are continually being bathed in "new" sea water. In the previous experiment it was found that a 25% loss did occur from the cells but, while the dividing cells remained in the same medium, there was no further loss to the medium. In this experiment *Nitzschia* cells containing Ce^{144} were suspended in nonactive medium for 30 minutes, centrifuged from this medium, and the process repeated three times. There was about an 8% loss from the cells during each of the four washings (Table 12).

Cells resuspended in culture medium containing a chelating substance. The loss of Ce^{144} from *Nitzschia* cells resuspended in culture medium with Versene was compared to that from cells in culture medium containing no added Versene. The *Nitzschia* cells were made radioactive with Ce^{144} by growing them for five days in culture medium containing 7.9 microcuries of Ce^{144} per liter. These cells were centrifuged from the active medium and resuspended in nonactive medium. This culture was divided into two cultures and 0.5 g of Versene per liter was added to one. Samples of 25 cc were removed from each culture periodically. The cells were centrifuged from the medium, and the activity lost to the medium was measured. There was a greater loss of Ce^{144} from cells suspended in medium to which Versene had been added (Table 13).

DISCUSSION

Even though the composition of fallout which descends upon land is the same as that which falls upon the oceans, its uptake by terrestrial plants is quite different from that of marine planktonic algae. For example, the radionuclide, Cs^{137} , which occurs in very small amounts in fallout, is responsible for most of the radioactivity found in the edible portion of terrestrial plants after the soil has been contaminated by fallout (Weiss *et al.* 1956). Planktonic algae, as will be reported in detail elsewhere, concentrate Cs^{137} only 1 to 3 times over amounts present in sea water in which they are grown. Also there is a difference in the uptake by terrestrial plants and planktonic algae of the radionuclide Ce^{144} , which occurs in large amounts in fallout. It has been reported that only negligible amounts of Ce^{144} are taken up by terrestrial plants (Neel *et al.* 1953), while in this investigation it has been shown that planktonic algae concentrate Ce^{144} from 3,000 to 4,500 times.

Why does Cs^{137} occur in such large amounts in the plants when present in such small quantities in the soil, and why do the rare earth elements occur in the plants in such small amounts when present in such large amounts in the soil? This difference in uptake may not necessarily indicate any difference in nutritive requirements for these elements, since the uptake of fission products

from the soil by plants appears to be a function of their solubility (Larson *et al.* 1953). Insoluble particles resulting from the bomb blast may not readily migrate through the soil and therefore could not come in contact with the roots. Weiss *et al.* (1956) do not agree with the argument that Cs^{137} was made available to the root system of the plants in greater concentration by preferential translocation through the soil. During a one-year interval between two measurements they found that the amount of Cs^{137} in relation to other nuclides in the upper layer of the soil remained essentially unchanged. It is our belief that this observation can partially be explained on the basis that the Cs^{137} and other soluble radionuclides have the longer half-lives; thus the greatest loss is from leaching. The rare earth elements and other less soluble radionuclides have much shorter half-lives, making the greatest loss due to decay. It could, theoretically, be possible that the loss due to leaching might equal the loss by decay, giving no apparent change in the relationship of Cs^{137} to other radionuclides. It still remains an indisputable fact that a radionuclide can not be taken up from the soil by a terrestrial plant until it occurs in the root zone area.

Cerium-144 has been found to occur mostly in the particulate state in sea water, and it was further demonstrated that dilution, temperature, salinity, and added cerium carrier all influence the amount of Ce^{144} occurring in the particulate state following its addition to sea water. Since Ce^{144} occurs as particles in sea water, the measurement of the rate at which it became associated with phytoplankton required that cells be separated from sea water by centrifuging.

Since Ce^{144} added to sea water occurs in both the ionic and particulate states, there may be at least two methods by which it becomes associated with phytoplankton cells. Ionic Ce^{144} was taken up by *Nitzschia* cells either by being taken into the cells or being adsorbed on their surfaces. Particulate Ce^{144} became associated with the *Nitzschia* cells as a result of particles and cells coming in contact with each other and sticking, as confirmed by the continued uptake of Ce^{144} by non-dividing cells kept in the dark.

That particles do become associated with phytoplankton cells has been known for several years. Harvey (1937) found that particles of ferric hydroxide collect on the surface of diatoms. He believed it very unlikely that this coming together of ferric hydroxide particles and phytoplankton was the result of electrostatic attraction. He suggested that the particles "just stick" when coming in contact with the cells, which in many instances have a mucilaginous-covered surface. Also, Goldberg (1952) demonstrated that particulate and colloidal forms of iron are utilized in the metabolism of *Asterionella japonica*.

The mixing of water in the oceans would bring the phytoplankton cells and any added Ce^{144} particles into contact more frequently than occurs in standing cultures in the laboratory, resulting in a more rapid uptake. In the laboratory *Nitzschia* cells in cultures shaken continuously took up a larger percentage of the Ce^{144} from the medium than cells in identical cultures not shaken. However, the loss of Ce^{144} by adsorption to the sides of the flask in the shaken cultures was so great that cells in the unshaken cultures actually contained more Ce^{144} than those in the shaken cultures.

It has been suggested by several investigators that phytoplankton are not important in the removal of radionuclides from sea water when these nuclides occur in the particulate state, such as following a bomb explosion. Their opinion is that zooplankton, being filter feeders, concentrate the activity by filtering the radioactive particles from the water. In this investigation it has been shown that phytoplankton can rapidly concentrate added Ce^{144} when it is present in the water as particles. In 24 hours different species of phytoplankton were found to have concentrated Ce^{144} from about 2,000 to 4,500 times over that in sea water. In contrast we found that adults of a copepod species, *Tigropus californicus* (Baker), in 24 hours concentrated Ce^{144} only 76 times over sea water concentrations, while adults of *Artemia salina* (L.) concentrated Ce^{144} about 167 times over sea water concentrations in the same period of time (experimental results to be reported elsewhere).

Since these experiments were conducted

for only 24 hours, one might suspect that the concentration factor for cerium in zooplankton would increase with time, while a decrease might occur in the concentration factor of the phytoplankton. We believe that this reasoning would be incorrect since zooplankton, if similar to other animals, do not metabolize the rare earth elements to any great extent. Most of the Ce^{144} in the zooplankton would either be in the filtering mechanism or in the gut. Thus, there would probably be no long term retention of the particles by the zooplankton unless the Ce^{144} was continuously available. Also, the number of Ce^{144} particles in the water would decrease with time as a result of dilution by the mixing of the water and by accumulation by living organisms, so that it would be more and more difficult for the zooplankton to accumulate radioactive particles from the water. As for the phytoplankton, it is believed that the amount of Ce^{144} concentrated by the cell would be controlled by the division rate of the cells in relation to the Ce^{144} remaining in the water. If the uptake of Ce^{144} by phytoplankton were lessened due to a reduction in the availability of Ce^{144} in the water, the amount of Ce^{144} per cell would be controlled more by the rate of division of the cell. Thus, there is no reason to suspect that the Ce^{144} would decrease at a faster rate with time in phytoplankton than in zooplankton.

Steel and Gloyna (1955) followed the uptake of Ce^{141} by algae under laboratory conditions in experimental oxidation ponds. From these studies they concluded that radioactive cerium will be completely removed from the water in the ponds, if conditions are suitable for growth of the algae and the ponds are properly operated.

In the present investigation it was found that a *Nitzschia* culture started with a relatively small number of dividing cells would remove all the Ce^{144} from the sea water medium in 16 days. In this period of time a single dose of Ce^{144} added to the oceans would be rapidly diluted by currents and the mixing of the water. Probably this dilution would occur at least as rapidly as the phytoplankton could concentrate the Ce^{144} . In experiments reporting the uptake of Ce^{144} by various population sizes of

Nitzschia it was shown that the number of cells required, in laboratory cultures, to remove all the Ce^{144} from the medium was many times larger than populations ever encountered in nature. Other uptake experiments not included in the experimental results with natural population sizes of plankton collected in water taken about 3 feet below the surface off the laboratory dock revealed that from 10 to 24 % of the Ce^{144} added to this water was taken up by the plankton in $\frac{1}{2}$ hour. From 44 to 54 % was removed if the plankton collections were placed in the light in the culture cabinet for 24 hours. Natural populations of plankton in water taken 5 to 10 miles offshore removed not over 7 % of the Ce^{144} in $\frac{1}{2}$ hour and only 22 % in 24 hours.

If radioactivity were added to sea water in a single dose and large amounts of it were rapidly taken up by the phytoplankton cells, these cells would be very important in passing the activity to higher trophic levels. However, it has already been demonstrated that time which would allow for dilution is necessary in the removal of Ce^{144} from sea water by planktonic algae. In the oceans other factors, with time, would reduce the amount of Ce^{144} in the water, so that it is difficult to assign the overall relative importance of phytoplankton as a potential concentrator of Ce^{144} and supplier of it to higher trophic levels. However, 9 to 10 months following the nuclear tests in the Pacific, radioactivity was found associated with plankton in the upper water mass (USAEC Operation Troll).

Also, after the radioactivity has been completely removed from the water by one process or another, the phytoplankton with the passage of time become less and less radioactive and thus are not as important in getting their contained activity into higher trophic levels. This is due to "biological dilution" or the reduction by cell division of activity per unit weight of protoplasm. If conditions are suitable for rapid cell division the radioactivity can be drastically diluted in a relatively short period of time, since a 50 % reduction of activity per cell occurs with each division.

Actually it is not necessary that the activity be completely removed from the water

before "biological dilution" comes into operation. Soon after the addition of a single dose of activity to sea water, it is reduced to a concentration where enough is not available to maintain the same rate of uptake. If cell division continues at the same rate or increases, then "biological dilution" occurs.

Since phytoplankton can concentrate Ce^{144} , different food chains or webs might be involved in getting this nuclide to higher trophic levels than would be expected if zooplankton were the primary concentrator. It can be concluded that phytoplankton are important in passing particles of Ce^{144} from the water to higher trophic levels.

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Lake Water and Sediment

V. Oxygen Consumed in Water over Sediment Cores¹

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ABSTRACT

In defining the ability of a lake to produce a crop, one begins by taking a unit area for measurement, which is tantamount to saying that productivity is proportional to area. The productivity per unit area, P , has been stated by Hayes to be inversely related to depth, being $\propto \sqrt{\frac{5}{m}}$ where m is the mean depth in meters. P will have to be multiplied by a factor K which defines the crop, whether fish, plankton, etc. It is further necessary to recognize that lakes, like farms, are sometimes on good land, sometimes on poor. This important variant may be given numerical expression as a quality index, QI , which is supposed to tell what a given lake could produce if it were considered as of standard depth 5 m. QI was previously determined for a number of lakes on the basis of fish population, factored for length of food chain. It is made to fluctuate around unity so that K is given a value of unity for fish. Finally there is a reducing correction b to be applied to certain measurements on bog lakes, which have, for example, high oxygen consumption and bacterial counts but support few fish. The bog factor may be a function of color. The foregoing considerations lead to the equation

$$P = K \sqrt{\frac{5}{m}} \times QI \times b$$

If a measurement were made on fish in a lake of 5 m depth, P would be the same as QI , all other terms having become unity.

Measurements are given of the oxygen consumption of water over mud in undisturbed profundal Jenkin cores from 16 east coast Canadian lakes. These are compared with artificial cores made by plunging a Jenkin tube into the mud of a dredge sample, and also with surface mud packed by centrifuge in bottles. All three methods gave the same values. On four lakes the field hypolimnetic deficit was compared with the laboratory tests. The mean oxygen consumptions were indistinguishable, but the field results did not arrange the lakes in the same order as laboratory tests and are judged to be related to basin dimensions rather than to productivity. Laboratory tests appear to be more accurate than field tests and of wider applicability, *e.g.*, to unstratified lakes or bog lakes. In laboratory tests a water blank is subtracted so that consumption can be obtained for mud surface only.

For 9 lakes a regression is calculated between oxygen consumption and QI for fish. The relation is highly significant and is given as

$$QI = 66.9 \times \text{oxygen consumption} - 1.31,$$

where the units of oxygen consumption are mg used per cm² mud surface per day.

It would appear to be a truism to say that the productivity of a lake is proportional to its area, which is equivalent to saying that a large farm will produce more than a small one, quality being equal. Rawson (1955 and earlier) considered the less obvious ques-

tion of how productivity is related to depth, and concluded that in large lakes the plankton and fish decline as depth increases. Hayes (1957) claims to have shown that the following relation describes the generality of lakes.

¹ This work was supported financially by the Nova Scotia Research Foundation and the National Research Council of Canada.

$$\text{Productivity per unit area} = K \sqrt{\frac{5}{m}}$$

where m is the mean depth and K depends on the units selected, whether plankton, fish, etc.

Having made allowance for lake dimensions, the next thing is to consider the quality of the producing mechanism, for which the mud surface could serve as a useful indicator, since it receives the fall-out resulting from photosynthesis above, and might be expected to support a suitable crop of micro-organisms to effect mineralization. The quality of the mud has been estimated from its oxygen consumption, determined by field measurements of the hypolimnetic deficit of Strøm which is discussed below. In this paper we are examining the utility of laboratory methods of measuring mud oxygen uptake. If applicable they are considerably less time-consuming and also probably more accurate than field tests.

Neither field nor laboratory methods will be expected to describe correctly the relations of bog lakes. These, although of low productivity, consume oxygen at a high rate because of organic matter in suspension and solution. Probably a bog correction should be introduced. It might be a function of water color, the method of allowance for which is indicated in Paper VI. In summary

$$P = K \sqrt{\frac{5}{m}} \times QI \times b$$

where QI is a quality index greater or less than one, and b is a bog factor, usually less than unity, to be applied as a reducer of certain secondary measurements of productivity in bog lakes.

The present paper suggests a preliminary equation between mud oxygen demand and fish, that is, it attempts to extend the meaning of QI . Consideration of b , the bog allowance, is deferred until later.

METHODS

The work to be described was carried out in the summers 1953 to 1957. For the first two summers the Jenkin sampler was used exclusively. Then the dummy-Jenkin or dredge-Jenkin, for which the field work is easier, was introduced for comparison. In 1957 the bottle tests, which further simplify

collection and transportation of mud, were begun. There are 3 lakes, Silver, Black Brook, and Southport, on which all three methods have been tried. On 12 lakes both real and dummy Jenkin tests are available, and on 4 lakes there are only bottle tests.

We shall first describe a Jenkin core experiment. The apparatus is illustrated by Mortimer (1941-42), and the tube as removed for preliminary aeration is shown by Hayes (1955). Upon collection it contains an undisturbed core of lake mud with clear lake water above it. Cores were brought to the laboratory in a portable refrigerator, and kept in a cold room. Six tubes were used at a time for two experiments, three tubes to each experiment. Each tube, as shown in Figure 1, is set up against a vertical heating unit, bent from a lead G.E. garden soil heater.

After the tubes were placed on the stand, 2 hr were allowed for temperature equilibration; then the water was aerated for 2 hr without disturbance of the mud. At the same time and temperature some topping water was under aeration. Eventually the tubes were stoppered to exclude air bubbles, which is easily accomplished by leaving a hole in the stopper, subsequently to be plugged by a glass rod.

All tests were conducted in the dark.

Two points were obtained from each tube, or six points per experiment. Call the tubes A, B, and C and imagine they are set up on Monday. On Tuesday tube A is opened, and an aliquot of water taken for duplicate oxygen determinations. The sample is replaced by aerated water, and the new O_2 content of the mixture is calculated. On Wednesday tube B is opened, on Thursday tube C. On Friday tube A is again tested and discarded, and so on. When rapid O_2 utilization occurred, 10- or 12-hr periods were used instead of days. Blanks were set up in the same way, using lake water only, without mud.

Oxygen was determined in test bottles by the Winkler method. The volumes of Winkler bottles and dimensions of tubes with or without mud were all known. Thus the results could be expressed as mg oxygen used up per cm^2 of mud surface. Blank

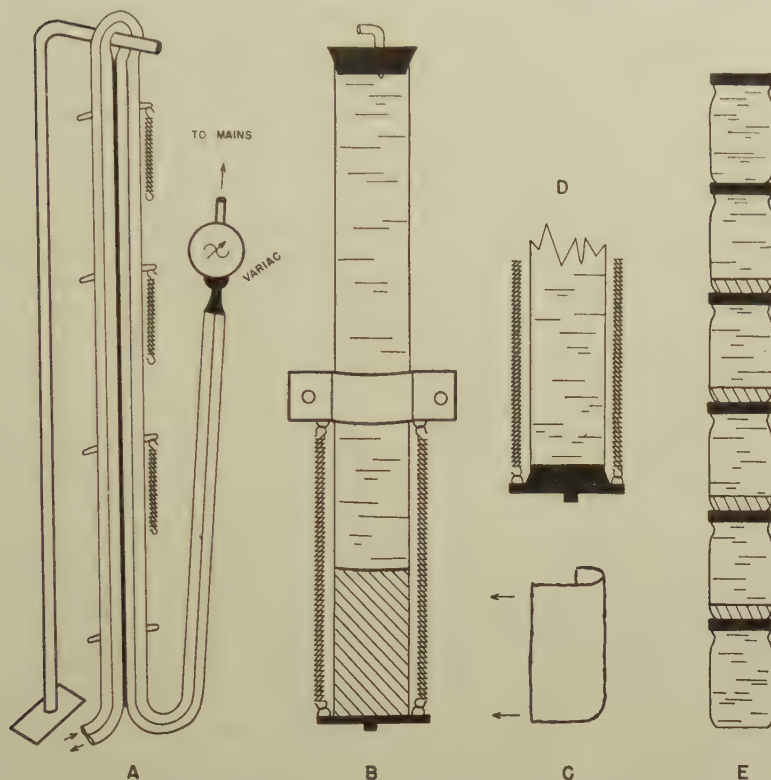


FIG. 1. Arrangement for measuring oxygen consumption over mud.

A. Heating unit bent from a General Electric garden soil heater drawing 400 watts. The double coils are bent up into six vertical pieces, one of which is illustrated. The loops are soldered together and held at the top by an iron stand secured to the table. They are fitted with springs by which a Jenkin tube is secured in place against the heated coil. The backs of the coils and exposed places are packed with asbestos. To test the stirring effectiveness of the heater, tubes were set up and dye added to the bottom. A ribbon of dye went up the tube adjacent to the heater and fanned out at the top and down the other side more diffusely. A variable transformer is used in order to secure mixing with minimal heating. It was found that 60 volts was adequate.

B. Jenkin tube after removal from the collecting apparatus, ready for a test. The bottom consists of a rubber pad on a brass disc, held in place by springs. The brass belt at center fits into the field apparatus. When oxygen measurements are in progress the top is closed by a rubber stopper with a hole in the center to allow water to escape when filling; the hole is plugged by a glass rod. An artificial Jenkin made up from dredged mud would look like B save for closure by a rubber stopper at bottom as well as top.

C. Sheet of asbestos webbing cut to the height of the mud core in a Jenkin tube. The sheet is placed between the tube and the heating coils to prevent the mud from heating excessively, with consequent danger of bubbling, or other disturbance, or increased oxygen consumption.

D. Tube set up for blank test, filled with water alone. Note rubber stopper at bottom, held in place by the springs.

E. Small bottles, as used in later tests, arranged to be placed against the heating coils. The water in bottles became a little warmer than the long tubes, and the mixing was not as effective. The four central bottles are shown as containing mud, and the top and bottom ones as water blanks.

values could be in the same terms or as utilization per litre.

The final expression of an experiment was a straight-line graph similar to the lower curve of Figure 2, from which the slope and

error of the line were calculated (Fig. 2 however is from a bottle experiment).

In the second method, called dummy- or dredge-Jenkin, a bucket of mud was collected with an Ekman dredge. The Jenkin

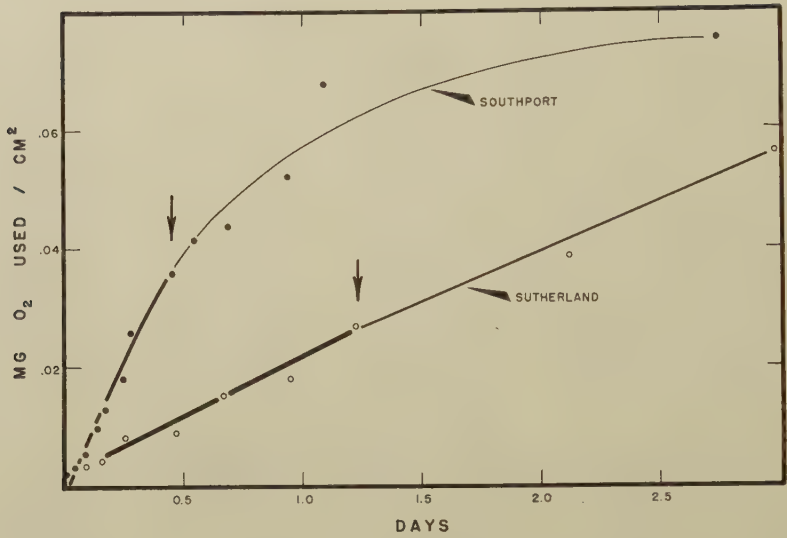


FIG. 2. Two illustrative mud curves in bottle experiments from which the blank has already been subtracted. Vertical arrows indicate the final point used to calculate the O₂ consumption. Heavy lines are calculated by the method of least squares. Southport pond illustrates well the diminishing rate of oxygen uptake, which was observed in nearly all experiments. It was minimal in Sutherland Lake. The cause of the levelling off might be stagnation over the mud due to inadequate stirring, or a slowing of uptake as the oxygen tension falls.

TABLE 1. *Oxygen used, mg per 100 cm² mud per day*

The dredge Jenkin, which took deeper mud gives lower readings. The ponds are artificial ones located in Prince Edward Island. Bottle experiments, in which only the top 5 cm of mud were taken, gave results indistinguishable from true Jenkin cores.

Lake	True Jenkin	Dredge Jenkin	Bottle
Montague	10.63	3.01	—
Southport	9.21	4.34	8.44

tube was plunged down into this mud and stoppered. Clean water was carefully run in and the procedure carried out as before. These results were indistinguishable from real Jenkin cores, except in the P.E.I. artificial ponds. Here the dummy Jenkins were notably lower, as Table 1 shows. By the time we got to the bottle tests, further precautions had been introduced, and only the top 5 cm of mud were taken. The oxygen consumption then read about the same as the true Jenkin.

In general we recommend that only the top 5 cm of mud be taken for artificial systems. An even thinner layer, if convenient,

is better. What we do is to decant the water off a carefully taken dredge sample and spoon the surface mud up into a jar. The limit at 5 cm is based on the results of bacterial counts to be discussed in paper VI (Hayes and Anthony, 1959).

Turning now to the bottle experiments, the bottles used were screw-cap cylinders, 8 cm high and about 5.65 cm inside diameter. They were selected to fit into the milk bottle compartments of a centrifuge. The area of the mud layer introduced into the bottom was 25 cm². These are the bottles illustrated in Figure 1, E, which shows them stacked. After the mud was introduced the bottles were centrifuged to pack it, and the supernatant fluid was decanted. Fully aerated surface water from the lake was added without disturbance, and the bottles were capped under the same water to exclude air bubbles. The bottles were placed, one on top of another, against the same heating coils as used for the Jenkin tests. From time to time a bottle was opened and a sample siphoned into smaller glass-stoppered bottles for Winkler tests. Results were brought to the same units as with the Jenkin tubes. The oxygen declined more quickly

in the smaller water volume so that half a day or a day sufficed to complete a series. Any number of bottles could be used including a series of blanks.

Figure 2 shows bottle tests on two lakes. The line for Southport, a rapidly consuming system, flattens to the right, indicating perhaps that the stirring was inadequate so that there was not enough fresh water circulating to meet the demands of the mud. This was observed in several lakes. The rate given has in all instances been taken from the initial, straight line part of the graph, as heavily marked in Figure 2.

The temperature at the interface reached about 12–12.5° in the bottle experiments. In Jenkin tubes there was a gradient from bottom to top of the long tubes from about 9° to over 13°. Probably the correct overall temperature to quote is $11 \pm 1.5^\circ\text{C}$.

For 1958, a better stirring arrangement was devised and preliminarily tested. Bottles of the kind illustrated in Figure 1, E, were provided with 2-hole rubber stoppers through which a 60 cm long inverted U-tube was passed, with one leg just above the mud and the other just below the stopper. A knife-type heater was bound against the longer leg with foil so that the local heating would create a water current inside. Such a heater, placed horizontally and controlled by a variable transformer, could stretch across three bottle systems. For initial clearance of air bubbles the top of the inverted U had a little outlet tube blown on which could be closed by rubber tubing and pinch cock. The bottles themselves were immersed in the water of a soft-drink cooler held at 11°C.

As a further modification towards uniformity a relatively inactive standard water was used, instead of water from the test lake. This was taken from Halifax area lakes which have approximately the same composition as Bluff Lake water.

Turning to 1958 results, the blank values agreed very well with previous Bluff Lake water tests, but the mud tests ran lower, occasionally down to half those of previous years. They will not be cited, since more work on the method is evidently necessary.

TABLE 2. *Summary of oxygen consumption results*
Lakes are arranged in the same order as in paper I, where they are characterized. Column 3 gives the number of experiments, each one representing a complete series of six or more tubes or bottles from which a graph showing consumption was made. Usually each test represented a visit to the lake, although occasionally one visit yielded samples for both real Jenkin and dummy Jenkin cores. Visits extended over five summers. Column 4 shows values attributed to mud surface alone after subtraction of blank. Column 6 gives water blank as per cent of the total or initial reading for mud plus water. Thus, e.g., the Punchbowl with a blank of 50% showed equal consumption for mud and water. For purposes of calculation the blanks were brought to the same terms as the mud, i.e., mg 100 cm², which is of course a fictitious unit. Column 8, quality indices are from Hayes (1957).

1	2	3	4	5	6	7	8
No.	Name	No. of tests	Mg O ₂ used/cm ² /day × 100	Standard error of col. 4 as %	Blank as % total reading	Field hypolimnetic deficit. Units as in col. 4	Quality index for fish
1	Bluff	4	1.88	17	39		
2	Punchbowl	5	4.08	9	50	1.65	
3	Silver	4	2.01	13	44		
4	Boar's Back	1	3.48		36		0.20
5	Jesse	2	3.17	16	35		0.53
6	Tedford	1	4.60		16		0.98
7	Black Brook	3	2.90	16	35	2.12	
8	Copper	3	3.09	10	24	1.80	0.36
9	Grand	2	2.64	13	29	3.00	
10	Lily	2	3.99	4	11		
11	Sutherland	1	1.98		27		0.87
12	Crecy	2	1.49	40	70		0.28
13	Gibson	2	3.76	11	33		0.31
14	Kerr	2	1.65	33	42		0.09
15	Montague	2	10.63	20	18		6.18
16	Southport	3	8.95	10	11		4.88

RESULTS

Since no significant differences were observed between the three kinds of measurement, results of all are consolidated in Table 2, column 4. The range, from minimum to maximum is somewhat more than five fold. Column 6 shows the blank values, of water only, which were often large. Thus in the

Punchbowl the blank was equal to the mud value. These blanks are expressed in the same units as the mud, consumption per cm^2 . The Jenkin tube and bottle tests agree on blank values in these units, but if we move to the usual expression, namely mg O_2 used per liter, the bottle blanks are nearly 5 times as high as the Jenkin. This is not far from the ratio of Jenkin tube height to bottle height and suggests that the blanks were not caused entirely by consumption of oxygen within the whole fluid volume but were related to an area factor. Possibly material settles out of the water and O_2 is used up at the bottom of the bottle.

Two series of bottle blanks were done, using various bottle sizes so that the ratio of inside area in cm^2 to volume in cm^3 ranged from 1.20 to 0.25. There was a suggestion of increase in oxygen consumption per ml with increasing relative surface but no clear pattern.

Hypolimnetic deficit

This measure of lake productivity, which asks how fast the stagnant depths can use up oxygen during summer stratification, was proposed by Strøm (1931). Ideal in principle, it is tedious in practice, requiring accurate contouring of the lake as well as several visits during one or more summers. It is not reliable on unproductive lakes, in which oxygen is used up so slowly that leakage from surface layers invalidates the method. In very productive waters or bog waters on the other hand, the mud can remove adjacent O_2 faster than the slow stirring of the depths can renew it, thereby yielding unduly low results. In bog lakes there is also a large consumption of oxygen by the water itself. There are also many lakes that do not stratify in summer, to which the method is not applicable.

Our laboratory tests were set up in imitation of the hypolimnetic deficit, in the hope of overcoming these difficulties, and results are in appropriate units for comparison. Four of our lakes were stratified, and on these field measurements were made in the manner of Strøm. When the results (col. 7) are compared with column 4 they are observed to be of the same order of magnitude. Comparison of the four pairs by the t -test

shows them to be indistinguishable. Within the group the order is not the same, the lowest of the four by laboratory test (Grand) being highest by field test, and vice versa (Punchbowl). The field results illustrate the difficulties already mentioned. Grand Lake, deep and unproductive, will have oxygen driven out of its hypolimnion into the surface layers by summer warming, to give the apparent consumption. Punchbowl, an extreme bog type of thistle-tube shape is never completely mixed; at least we have never been able to observe full saturation of the depths during several spring turnovers. The mud surface is restricted in its use of oxygen by delivery failure, with the resulting low value for the deficit.

In summary, it appears that the laboratory tests are giving the same results as the field tests but with a higher level of accuracy.

A physiologist, if he were to read this paper, would no doubt wonder at the cumbersome oxygen consumption methods selected. It must be remembered that we entered this work with the hypothesis that the intact mud-water interface has some special properties, comparable perhaps to those of a cell membrane. These properties were not to be destroyed by gross disturbance, hence the use of a Jenkin sampler. Use of this apparatus permitted a test of the hypothesis, which, as it turned out, could not be sustained. In our experience the upper 5 cm of sediment can be rapidly stabilized in a bottle so that its surface reacts like that of an intact lake bottom. Our proposal for the future would be to use a suspension of surface sediment in a Warburg apparatus at 20° and, initially at least, to factor the results back to the terminology of Table 2.

We do have some evidence, not conclusive, that there may be a special interface produced in summer, following a plankton bloom and due to an unusually large, temporary, decomposing fall-out. Along the same line, Thomas (1955) made an interesting comparison on three lakes between the hypolimnetic deficit and the oxygen consumption of the falling sediments collected on submerged surfaces. The latter measurements were made in the laboratory at

room temperature, and calculated back to comparable units, including a subtraction to allow for the stream outflow. On each lake the two measures were virtually the same

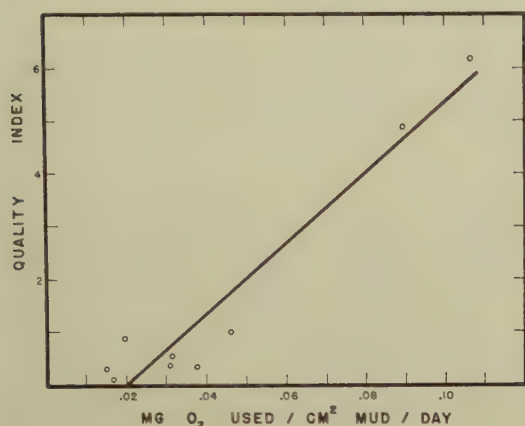


FIG. 3. Relation of oxygen consumed over mud surface to quality index. *QI* values from Hayes (1957) represent fish population, factored to allow for length of food chain of the different fish species. The line as drawn was calculated by the method of least squares. The relationship shown is highly significant. Boar's Back Lake is omitted from the graph.

so that either one might define productivity. The meaning is presumably that the hypolimnetic deficit is caused by the oxygen uptake of sediment, fallen and falling, during the period under study.

The same consideration would apply to field oxygen deficit studies by the method of Strøm, or to CO_2 accumulation (Ohle 1952). These will necessarily reflect largely the consumption of the current year with little carry over from one year to another.

To sum up: oxygen measurements are probably less reliable, as production indices, when made over the intact sediment surface than when mixed mud from the top few cm is used. The mixed mud might be taken as an integration of the performance of the lake during its recent years.

Quality index

For ten of the lakes used in this study the abundance of fish has been estimated either via angling returns or total population. Hayes (1957) has made allowance for length of food chain of the fish involved and for dimensions of each lake to produce a quality index, *QI*, which purports to describe the

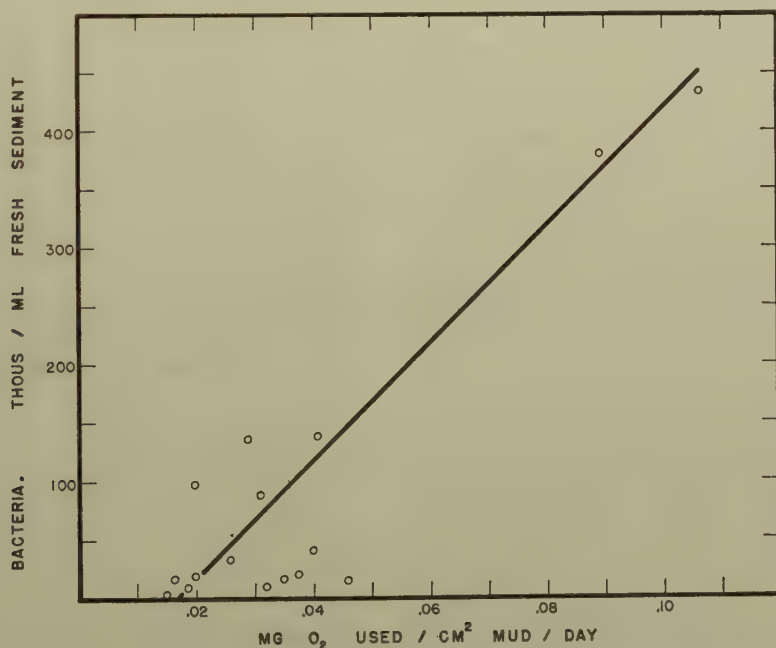


FIG. 4. Relation of oxygen consumed over mud surface to bacterial count per ml fresh surface sediment. Bacterial values from Hayes and Anthony (1959), paper VI of this series.

basic productivity of the lake (see Table 2, col. 8).

Calculation shows that there is a highly significant relation between columns 4 and 8. We do not feel that Boar's Back Lake properly demonstrates the relationship because it is an acid bog lake with highly colored water (paper I). Such lakes are low on fish but high on oxygen consumption. It turns out that 9 lakes (excluding Boar's Back) give a standard error of the regression line of ± 0.55 and a correlation coefficient of 0.97, while all 10 lakes do less well with ± 0.60 and 0.96 respectively. The 9 points and calculated line are illustrated in Figure 3. The relationship is

$QR = 66.9 \times \text{oxygen consumption} - 1.31$
The numerical values of the constants are based on too small a series of lakes to have much validity, but they indicate what will be forthcoming when more work is done. (At the suggestion of a referee column 4 of Table 2 has been multiplied by 100 for easier reading. The change has not, however, been carried through to Figure 3 or to the above equation.)

It is an unfortunate deficiency in our work, well illustrated in Figure 3, that we do not have an adequate productivity series. We have unproductive lakes, bog lakes, and the two artificial P.E.I. ponds. A longer series, chosen farther afield, will be necessary to produce numerical constants that can be trusted.

There are several columns in paper I, Table 1, which could yield a significant relationship to oxygen consumption, for example alkalinity, conductivity, and calcium. As above, these depend on the P.E.I. ponds at the high end and the rest of the lakes at the low end.

Bacterial count

In paper VI the bacterial counts on the sediments of the 16 lakes are given, and their

relation to oxygen consumption is shown in Figure 4. The line cuts the base line (zero bacteria) at 0.017 mg O₂. This intercept suggests that there is a possible consumption of oxygen unconnected with microorganisms, which might be called chemical uptake. Lönnerblad (1930) describes a marked uptake of oxygen by sterile bog lake sediments, less by those of balanced lakes.

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Lake Water and Sediment

VI. The Standing Crop of Bacteria in Lake Sediments and Its Place in the Classification of Lakes¹

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ABSTRACT

Procedures are given and field apparatus described for use of the millipore filter technique for lake water and sediment bacteria. Agar plate and filter procedures give the same counts using a supplemented sodium caseinate medium. Direct counts were higher than colony counts by factors from 59 to 233, with a mean of 111. Cultures made on lake sediment extract in agar were inferior to the caseinate medium in unproductive lakes, but when taken from productive lake soils they were adequate to produce colony growth. Several hundred quadruplicate tests were studied to establish errors, which had two components: the theoretical error of a Poisson series which depends on the number of colonies on a filter, and a handling error which is $\pm 9\%$. Combining the above errors with the natural variability of profundal sediment from station to station and visit to visit, and expressing the standard error of the mean as a per cent, the expectations are: one visit to take one core at each of four profundal stations comprising the top two or three cm will yield, on quadruplicate plates, an error of $\pm 130\%$. Hence, while a single visit may set a quite useful maximum it cannot be used to set a minimum. In the procedure adopted four field trips as above were made, by which the error was reduced to $\pm 36\%$.

Shallow cores, down to 15 cm or more, were sectioned. Surface scrapings did not yield the very high counts that others have reported. There was no significant decline in numbers down to about 5 cm, which comprises Naumann's "actual" layer. Below this a decline sets in upon entering the "historical" layer. Deep cores were taken on two lakes down to base gravel presumed 15,000 yr old. There was a decline with depth which appeared to be logarithmic, although the deep counts were too close to zero to prove it.

The authors' counts, supplemented by those of Henrici and McCoy, are plotted against other measurements thought to be related to productivity, namely methyl orange alkalinity, conductivity, and (in paper V of the series) oxygen consumption over mud. A good regression is observed in all clear-water lakes. However, brown-water lakes, although low in alkalinity and conductivity, have high bacterial counts. In these lakes there is a good correlation of bacterial count with water color. This provides a possible basis for stating what part of the bacterial count of any lake is to be attributed to bog. By subtraction of the bog fraction the real or useful measurement as indicative of productivity is obtained.

The ultimate measure of the productivity of a lake is presumably its ability to produce the crop in which man is interested. Most people (professional limnologists excepted) would probably name the crop as fish, which therefore becomes the current standard by which other estimates are judged. Other measurements will be of value insofar as they are economical to make and correctly indicate the ordinarily expected crop of fish. They will also assist in the interpretation of unusual conditions, or of operations in which the stock of fish is manipulated by man.

Lakes were classified by Naumann in terms of their nutritive level, which may be given as ill-nourished and well-nourished. Later a middle class was added, and then Thienemann added a class of disproportioned or wrongly nourished lakes. The last group may be further divided into those naturally bad (brown lakes, either bog or fen) and lakes made wrong through pollution caused by man. It has been suggested that since natural disproportion is approached in an infinite series of variations and gradations,

¹ The authors acknowledge with gratitude the support of the National Research Council of Canada and the Nova Scotia Research Foundation.



FIG. 1. Glassware for sampling bacteria. All bottles are supplied with bakelite caps which are discarded after one use. (1) Narrow neck 4-oz bottle to which about 40 ml of distilled water, adjusted to an ionic level equivalent to lake water, is added before sterilization; used for collection of water samples. (2) Same kind of bottle with graduation at 90 ml, made up to mark before sterilization and corrected for evaporation with sterile water just before use; used for dilution of mud samples beyond the initial dilution described in text. (3) Widemouthed 16-oz jars containing two dozen glass beads, filled to 250-ml graduation mark before sterilization and re-adjusted before use. These are for the initial dilution of mud samples. Inexpensive pint mason preserving jars have recently been found more efficient. (4) Wine bottles for rising, etc. are provided when used with a sterile delivery spout (4a). (5) Test tubes to take 20 ml medium. (6) Bicknell automatic pipette to deliver medium to filter pads. (7) Open tube with a mark so that the volume to

the term has no definable meaning as describing a type of lake (see Berg and Petersen 1956, and Ohle 1955).

The system outlined has been variously refined and has enjoyed wide use, the terms acquiring, perhaps, an apparent enhancement in precision from the general custom of rendering them in Greek.

The quantitative basis for productivity might be related to two processes. The first is the provision of organic matter, of which the sources are current photosynthesis and previous photosynthesis through drainage. The second is the destruction or mineralization of organic matter. In a balanced lake the two processes are in equilibrium, so that a measure of either one might be expected to indicate correctly the crop of paramount interest, *e.g.*, fish. Chemical analyses are also suitable indicators.

When lakes are out of balance, *i.e.*, disproportioned, there is more organic matter coming in, either by nature (brown lakes) or by drainage related to activities of man, than can be equilibrated by the break-down system. The secondary standards which work quite well with balanced lakes will no longer serve. Take for example bacteria. In a balanced lake their numbers in the sediment could conceivably be brought into relation with the fish crop. In a brown water lake, bacteria might be expected to be high, in an effort, so to speak, to mineralize the extra organic matter. The fish crop in such lakes is notably low. Now if bacteria, as characterizing the mineralizing capacity, could be equated with other measures which characterize the synthetic capacity, and brought into relation with the chemistry of

mark is known. The tube is plunged to the mark, or to a measurable depth, into an Ekman dredge sample. The lower, then the upper sterile stopper is inserted and the tube is brought back to the laboratory under refrigeration. Eventually its contents are delivered into jar (3). (If desired the transfer may be made at once and subsequent operations carried out in the field using apparatus shown in Fig. 2.) To our surprise no increase in counts was detected even after several days' storage. After a fortnight or more in storage, marked increases were sometimes observed. Tubes similar to (7) are used to obtain mud samples for weighing wet and dry; from this the dry weight count is obtained.

the water, an overall index might be established which would be in true relation to the crop of fish. The same series of measurements should ultimately apply equally well to clear-water and brown-water lakes.

The meaning of bacterial counts is as important in terrestrial soils as underwater ones. In the former the local variability of conditions is greater because of seasonal changes, moisture, and particle size as well as addition of fertilizer. Even so the numbers of bacteria including actinomycetes has been significantly correlated with organic matter in field plots after annual treatment with straw and phosphate (Gray and Wallace 1957).

These were the considerations which led to the present investigation. The first necessity was to establish the reliability of bacterial counts. The next was to determine the relation of bacterial numbers to other information about several lakes of differing character. The results to date follow.

PROCEDURE

In this section numbers in parentheses refer to pieces of glassware shown in Figure 1. Unless otherwise indicated, all counts quoted were obtained by use of so-called molecular filters, trade name "Millipore." Molecular filters were introduced 100 years ago by Fick (Ferry 1936). Their accuracy, when tested on terrestrial soils, is 3 to 5 times that of agar plate counts (Davey and Wilde 1955).

All of the sediment samples were taken in the deep, open parts of lakes, never in coves or shallow bays or at the inlet or outlet. In general practice lake sediments are sampled with an Ekman dredge. The Ekman sample is inspected to see that the surface has not been disturbed. Free water above the sample is siphoned off. The mud sample for bacterial count is taken by a tube (7) plunged vertically into the surface and brought to the laboratory under refrigeration. An examination of sections of Naumann cores indicates that bacteria are equally distributed in at least the upper 5 cm of lake profundal sediments. Small disturbances of the surface of Ekman samples are probably not significant.

The first dilution of mud is to 250 ml

when tube (7) is emptied into jar (3). One further dilution is made, by adding 10 ml from (3) to bottle (2). One ml is taken for culturing, diluted by adding to roughly 40 ml in bottle (1), and poured through a filter. This deposits nearly the maximum amount of mud on the filter through which colonies can be readily seen. With the least fertile of our lakes only a small number of colonies, say two or three, came up on a filter. The errors from averaged quadruplicates at this level are large (Fig. 3), and this determines in practice the limit of the Millipore method. As the granite-slate-quartzite lakes in our area are probably about as infertile as any to be found in the temperate zone, it may be said that the Millipore method will just suffice.

Samples to be sectioned for determination of vertical distribution are collected with a Naumann sampler (Welch 1948, p. 183) and the tubes transferred to a portable refrigerator. Storage up to a fortnight has not produced observable increase in counts. The sample is eventually forced out of the tube and sections taken for dilution as described and illustrated by Hayes (1955).

For counts on water, the sample is brought up from the desired depth with a Kemmerer bottle, and one ml or 10 ml is pipetted to a dilution bottle (1). Filtration is carried out in the field as soon as practicable, at all events within about an hour. Even for short intervals refrigeration is advisable. Solid carbon dioxide wrapped in newspaper is a good 24-hour refrigerant. The field kit shown in Figure 2 is used for water, and on extended trips it may also be desirable to use it for mud samples.

Filtration and counting techniques are described in trade releases of the Millipore Corp. Incubation at room temperature takes two or three days, during which time the dishes are kept in a plastic bread box with wet blotting paper at the bottom.

No attempts have yet been made to identify the colonies which come up to be counted. We have, however, had occasion to make numerous tests with antibiotics including terramycin and later tetracycline. The effect was a total kill. It is therefore concluded that we are reporting here on bacteria rather than yeasts or moulds.

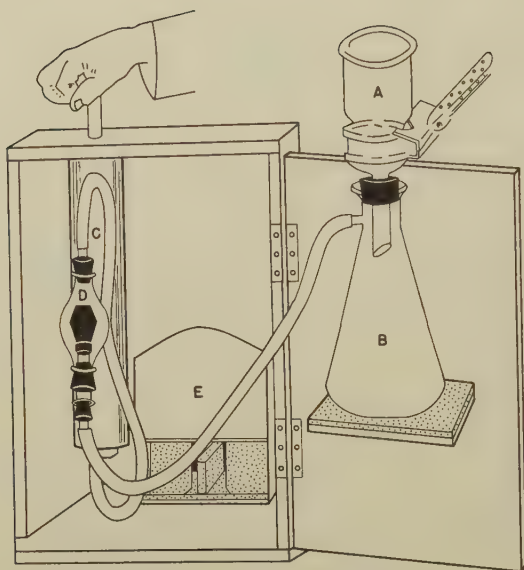


FIG. 2. Field kit for Millipore sampling. The whole apparatus is arranged in a box to withstand breakage in transit. *A* is a Millipore head in which the filter is placed and through which the sample containing bacteria is drawn by suction into the flask *B*. The flask is emptied from time to time. The necessary suction is applied at *C* by a rubber-boat pump with valve reversed. A flutter valve, *D*, is put into the circuit to prevent loss of suction when pumping is stopped. There is a compartment lined with sponge rubber and provided with a sliding door, *E*, for storage of the filtering head.

Pigmented bacteria are abundant in lake waters (Snow and Fred 1926). Laboratory water in aquaria, etc. may yield lemon-yellow, pink, or occasionally purple colonies. As to mud, Henrici and McCoy (1938) report 10 per cent or more chromogens from averaged Lake Mendota samples, which would include shallow stations in coves. In terrestrial soil, pigmented bacteria are rare. In our own sediment samples, which were all taken from the deep parts of lakes, colonies were nearly always white to ivory, with chromogens ranging from zero to scarce. Once or twice however, chromogens have dominated a culture. After some consideration it was decided to subtract chromogens from all totals to be reported. This involves an assumption, which we hope is correct, that the colorless colonies give the best numerical indication of the indigenous profundal population. Also contaminants, ei-

ther from the air or from the descending sampler, are likely to be chromogens. Spreading moulds, which were infrequently seen, were also omitted from reported totals.

Many hundreds of anaerobic counts have been made in the sediments. Only those from the most ancient levels are considered in the results presented here. It takes about two weeks or more, instead of two or three days, for the colonies to grow large enough to be counted. During the time of growth, necessary periodic inspections involve difficulties in maintaining anaerobic and moisture conditions. The scatter in replicates is large, and frequently nothing appears. Possibly added precautions which are planned for future work will make the method satisfactory.

Counts of surface water bacteria have been made at intervals on all lakes. The scatter is considerable, and from collections on some trips no colonies at all come up. So far the water counts have not been brought into any quantitative relation to other productivity indices. Presentation of them is deferred pending further work.

COMPARISON OF AGAR AND MILLIPORE COUNTS

A comparison was made on the contents of 25 dilution jars (3) containing a mud sample. Five lakes were included. Quintuplicate plates or filters were taken, *i.e.*, a total of 125 of each. The average of all agar to Millipore ratios was 0.994, showing that the two methods give the same results. On another comparison, the earlier (prior to 1953) agar counts on five lakes where Naumann tube sections had been made, were compared with the quoted Millipore values in Table 3. The mean ratio was 1.2, also indistinguishable from unity. Thus it appears that results of the two methods are interchangeable where the same type of medium is used. It also indicates that there is no serious killing effect due to the heat of the melted agar.

DIRECT COUNTS

It is well known that direct bacterial counts are at variance with plate counts, giving results some two orders of magnitude greater. Some workers have felt that the

direct method gives a more correct picture of the soil conditions, others that the colony counts are better as indicating life, activity, and capacity to grow. It does not seem to be established whether one measure is, within error, a simple multiple of the other. If so the two will yield the same conclusions. In reviewing the problem Henrici (1939) says: "I began my work on lake bacteria with a prejudice against the plate count. As I have found the results of such counts to correlate with the results of microscopic counts and with other data, I have been forced to revise my opinion."

In our experience the direct count method is not decisively slower or less reproducible than the others. It is perhaps more trouble for a student to learn, and when used with water the bacteria must be concentrated before counting. We abandoned it mainly because of the impossibility of separating dead or dormant organisms from active ones.

Nine comparisons of direct and agar counts were made on sediments from five lakes. Direct counts were higher by factors of 59 to 233 with a mean of 111. This comparison is on the basis of directly counted groups, whether of one or several bacteria. Thus if a field showed a group of seven individual bacteria plus three separate cells, the number was called four. The thought is that the group of seven, if grown on agar, would come up (if at all) as one colony. The mean ratio of number of bacteria to number of groups was 1.4.

Kuznetsov (1958), using direct counts, reports that 10% of bacteria are dead in surface waters, 30% in surface sediment and 70% dead at 4 m sediment depth. The proportion of dead cells, by his method of differential staining obviously does not correspond to the 98-99.6% which failed to grow into colonies in our comparisons. The series of Russian lakes studied by Kuznetsov gave direct counts of 0.2 to 2×10^9 bacteria per gm fresh sediment, which is 3 or 4 orders of magnitude above our colony count range.

It is a commonplace to hear that some or even most kinds of microorganisms fail to grow on any given medium. The fact is illustrated by the practice in medical laboratories, of keeping 100 or more media on

the shelf, each designed to bring up a particular organism. So if we are here getting only a few of the varieties present as well as only one hundredth of the total number that can be seen, what hope is there of quantitative validity in soil cultures? A pragmatic answer is given in the final three illustrations of this paper, from which it appears that counts of bacteria are in fact related to other limnological measurements. Evidently we have a valid sampling procedure, equivalent to comparing the populations of towns by counting the policemen or grocers.

MEDIUM

A sodium caseinate medium was used. It was originated by Fred and his associates at Wisconsin and has been variously modified since (Henrici and McCoy 1938; Taylor 1940, 1951). Our current formulation is as follows: 0.2 per cent of glycerol; 0.1 per cent each of sodium caseinate, peptone, starch, and liver extract; 0.01 per cent each of MgSO_4 and CaCl_2 ; 0.04 per cent K_2HPO_4 , substituted as necessary by K_3PO_4 to bring final pH after autoclaving to about 7.0; and to each liter 15 gamma (one ampoule) vitamin B_{12} and a trace of iron (smallest pinch of FeCl_3 or FeSO_4).

In accordance with Millipore practice, the above is double the strength recommended for use with agar plates. Vitamin B_{12} was introduced because of its known value to soil bacterial cultures (Lochhead and Thexton 1951, 1952). It is also known to be required by marine diatoms, flagellates, and algae (Lewin 1954, Harvey 1955). There is besides, another soil extract required by some organisms. It is in nature a product of microbial synthesis, possibly folic acid, and for it liver extract may be substituted (Lochhead *et al.* 1952).

It is known that a good medium for soil bacteria may be prepared by adding soil extract to agar (Taylor 1951). This was tried. Equal weights of lake mud and water were autoclaved for an hour and filtered when cool. In Table 1 the soil results are compared with counts using caseinate medium. It appears that the sediment of lakes in the most inhospitable surroundings is inferior to a prepared me-

TABLE 1. *Comparison of colony numbers on agar plates made up with caseinate medium (but not including vitamin B₁₂ or liver supplement) and with lake soil extract*

The counting errors are about $\pm 10\%$. Comparisons are to be made horizontally but not vertically, *i.e.*, the lakes are not to be compared with one another on a basis of absolute numbers, as the sample sizes were not alike.

Lake	Lake pH	Character of lake	Medium at pH 7.3	Medium at lake pH	Medium + soil at pH 7.3	Soil at lake pH	Soil at pH 7.3	Soil count as % of medium count
Punchbowl	5.7	Acid bog in granite area	105		112	40		38
Bluff	5.4	Primitive, in granite area	94	98		50	42	48
Grand	6.6	Mostly unarable surroundings, some farm land	224	219		167	127	66
Lily	6.3	Near farm area	104	146		179	168	139
Copper	5.8	Farms	209	224		362	325	159

dium. Of those in arable territory the soil appears adequate. Differences in pH do not show any effect in final count, although the colonies take longer to appear under acid conditions.

Several variants of the above experiment were performed using the Millipore technique. Lake mud was suspended in Irish moss extract (carageenan), in which the finer particles remain stabilized for some time. Muds from Bluff Lake and the Punchbowl were added to Taylor's medium with and without vitamin supplements. Details will not be presented, but the conclusion was that mud supplements increased neither the size nor number of colonies. The vitamin supplements had no measurable effect on the number of colonies, but strongly stimulated their growth, leading to much larger colonies, or alternately making possible an earlier count. There was also a difference in tint, those without vitamins being dead white and those with the supplement tending towards ivory.

The accuracy of counting bacteria is not very high, so that a rather scarce group which might be brought up only in the presence of a special vitamin supplement could have been missed. For example Lochhead and Burton (1956) report that out of 14.1 million bacteria per gram of terrestrial soil, one million require nicotinic acid for growth. In our measurements these would have scarcely been perceived as an increment when the vitamin supplement was added.

ACCURACY

Unless otherwise identified the numbers quoted in this section are standard errors of the mean expressed as percentages of the mean, and obtained with the Millipore method. The values given refer to tests in quadruplicate. One obtains the standard deviation of the series and the error of the mean (using $n - 1$), which is multiplied by the appropriate t value, 1.25, from column 7, Table III of Fisher and Yates (1943). Occasionally, particularly in the matter of visits to lakes, the real number of tests was not four but some other number (say five). The final multiplication was then by an appropriate t value (say 1.19) and then by the ratio $\frac{\sqrt{5}}{\sqrt{4}}$ to find the theoretical standard error of quadruplicate tests.

We begin with counting errors, as when four one-ml samples are removed from the second dilution bottle (2) to make a 1:2500 part of the original mud. Results are given in Figure 3A, which is based on 309 sets from 16 lakes. There is a marked decline with increased sample size, levelling off at a little over $\pm 10\%$.

Bacterial counts theoretically constitute a Poisson series (Jones and Mollison 1948) in which the standard deviation squared is equal to the mean. On this basis the theoretical curve of error has been calculated, as shown in Figure 3B. It is of the same shape as the observed curve but lower, levelling off at about $\pm 3\%$. Now in a Poisson series it is presumed that all the error is due to the randomness of the distribution, the hu-

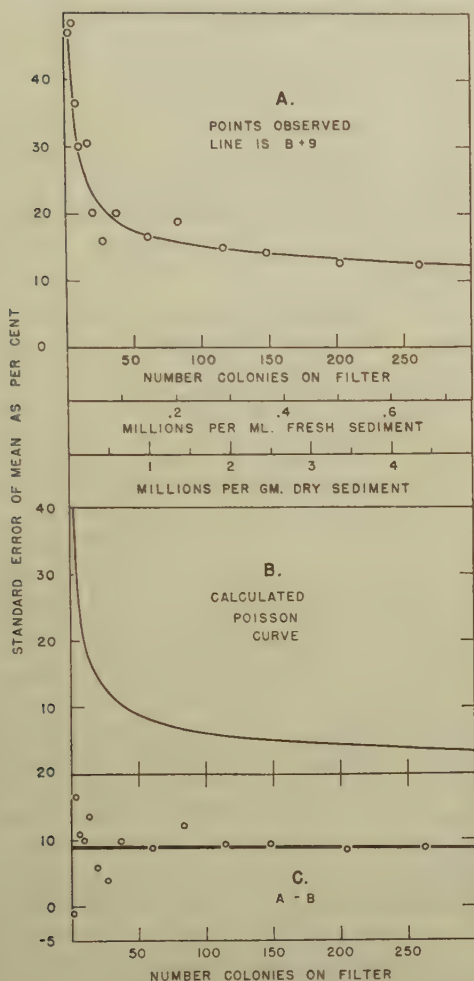


FIG. 3. Standard error of the mean in bacterial colony counts where quadruplicates are removed from the same dilution jar. In A each point is the mean of 20 tests. The line drawn is actually the line of curve B plus 9.0%; it is therefore in a sense a theoretical line. B is the calculated Poisson curve for quadruplicate samples, constructed on the presumption that the standard error is

$$\sqrt{\frac{M}{4}} \times 1.25.$$

C shows the subtraction of the Poisson curve of randomness from the observed points, and describes the error due to handling; it appears to be independent of the sample size.

The number of colonies per filter was the original form of observation and should have general validity. The conversions to a volume or weight basis of mud are approximate and apply to our particular technique; they would not be generally applicable.

man or handling or instrumental error being negligible. This is true of Geiger counting, but hardly of bacterial counts or even blood counts (Berkson *et al.* 1939). We might visualize the handling errors by naming just one of them, say the variation in volume of the one ml sampling pipettes. The A.P.H.A. specification for these allows a tolerance of $\pm 2.5\%$. The pipette error, as a fraction of the mean, will be independent of sample size. Hence its plot on Figure 3 would be horizontal.

The combined effect of handling errors is shown in Figure 3C, obtained by subtracting the Poisson curve from the observed points. These errors amount to 9.0% of the mean and leave a good deal of room for improvement in technique before the ultimate limit set by a Poisson distribution is reached.

Some further information on sampling accuracy is given in Table 2, which may now be discussed.

The mean error of all the observations illustrated in Figure 3A was $\pm 23\%$, showing that our mean colony count fell on the inflecting part of the curve. The original sample might be taken up from a dredge into a tube (7), or sliced from a Naumann core. In either case it was measured as a volume, which was converted to a weight as already described. The error of conversion, due mainly to the amount of water in a sample from the surface of an Ekman dredge, was $\pm 9.6\%$. Thus the combined sampling error in the number of bacteria per g dry weight is

$$\sqrt{23^2 + 9.6^2} = \pm 25\%$$

Next consider the collection of four sediment samples from the same lake station on a single visit. Four sampling tubes (7) might be plunged to the mark into the same dredge haul. One added error will concern the volume of the sample taken into the tube. We have no measure of this but it might be $\pm 10\%$. If the true count is assumed to be the same in the four samples, the total measuring error will be

$$\frac{\sqrt{23^2 + 9.6^2 + 10^2}}{\sqrt{4}} = \pm 13\%$$

TABLE 2. *Variability of counts taken in several ways*

Calculations are given in text. Except for columns 1 and 2 all numbers are standard errors of the mean expressed as a per cent of the mean and should be read $\pm$.

Number	Nature of sample	1 Number of samples	2 Number of lakes	3 Dilu- tion and hand- ling	4 Conver- sion to weight basis	5 Volume esti- mate of original mud	6 Com- bined previous columns	7 Ob- served total on this item	8 Col. 7 minus col. 6. Due to variation in material or not ac- counted for in handling.
1	Four one ml pipettes each repre- senting a mud-volume dilution of 1:2500	309	16	23					
	As above	187	13		9.6		25		
	As above, estimated					10			
2	Four mud samples, each treated as above, from the same lake sta- tion at the same time	6	2				13	31	18
3	Same as 2, but taken at different profundal stations	17	11				13	35	22
4	Same as 3 but with value derived from 5. Converts item 3 to real bacterial variability in lake, not just on this day but generally						13	130	117
5	Four profundal samples, one taken on each of four trips	44	11				13	71	58
6	Sixteen samples taken on four visits, four each time from dif- ferent profundal stations. Data derived from item 5						6.7	36	29

which is due to handling (column 6). Now the observed variability from all causes, as entered in column 7 was $\pm 31\%$. The difference between column 6 and column 7 of ± 18 may represent in part a real fluctuation, and in part handling technique, for instance variation in the shaking of the sampling jars (3), which affects counts (Jannasch 1956). We have also made counts where four samples were taken on the same visit, but from different profundal stations. The boat was moved between dredge hauls, and one core (7) was taken from each haul. The observed error was $\pm 35\%$, leaving a residual variation of $35 - 13 = \pm 22\%$. The two numbers 31 and 35 do not differ significantly, so that we are unable to discern any variability over the depths of a lake. (It might be repeated here that we did not take samples in coves or bays or near the inlet or outlet.)

The next question concerns multiple visits. When one sample is taken on each of four trips the standard error of the mean is observed as $\pm 71\%$. The presumed han-

dling error being, as above, $\pm 13\%$, we may take the real fluctuation in bacteria to be $\pm 58\%$.

It is of interest to work backwards from this last number to a guess at the accuracy of the number which would be quoted from quadruplicate samples taken on a single visit. It would be

$$58 \times \sqrt{4} + 13 = \pm 130\%$$

or to state it fully:

$$\text{mean} \pm 130\%$$

Hence, while a single visit may be expected on the average to set a quite useful maximum, it cannot be used to set a minimum. The mean plus a possible one or two hundred per cent would serve to characterize a lake as primitive, productive or polluted, and perhaps even more closely than that.

Turning forward again, consider finally the accuracy when four trips are made and each time four samples are taken. The

handling errors will be cut in half to

$$\frac{13}{\sqrt{4}} = \pm 6.7\%$$

The real fluctuation in bacteria will also be

TABLE 3. *Bacteria in the top 5 cm of sediment*

Lakes are arranged in the same order as in paper I where they are characterized. All stations are profundal, *i.e.*, not in shallow bays or littoral areas. The recent sampling procedure on a trip is to anchor a boat at four randomly selected open water sites. Thus "station" does not mean a precisely located spot. In trips of earlier years only one or two stations might be taken. As mentioned in the text, the differences from trip to trip were greater than the differences from station to station on the same trip.

Column 3 gives the number of field trips and column 4 the total number of stations. To obtain the bacterial count set down, a mean for each trip was obtained arithmetically. These trip means were averaged via logarithms. The method is illustrated in Table 5. The final column at right gives, as a % of the arithmetic mean, the reduction in value which resulted from using the logs of trip means to obtain the quoted final average, instead of taking a straight arithmetic mean. For example, in Bluff Lake the arithmetic mean for colonies per gm dry weight

$$\text{was } \frac{154 \times 100}{100 - 17.2} = 186.$$

1 Number	2 Name	3 Number of trips	4 Number of stations	Thousands of colonies bacteria in sediment		
				Per ml fresh	Per g dry	% red. via logs
1	Bluff	5	11	11	154	17.2
2	Punchbowl	5	14	121	1216	29.6
3	Silver	4	16	90	664	9.2
4	Boar's Back	5	20	22	218	17.4
5	Jesse	4	16	10	74	4.6
6	Tedford	4	16	15	115	36.6
7	Black Brook	5	14	134	1409	17.1
8	Copper	5	5	88	645	17.4
9	Grand	5	8	30	211	16.9
10	Lily	4	4	41	407	26.8
11	Sutherland	5	24	22	118	17.4
12	Crecy	4	14	9	108	64.1
13	Gibson	4	14	20	332	15.7
14	Kerr	4	14	22	262	17.3
15	Montague	2	5	433	1782	21.8
16	Southport	4	7	380	2079	26.7

expected to be improved in accuracy to

$$\frac{58}{\sqrt{4}} = \pm 29\%$$

The two errors together amount to $\pm 36\%$ on the quoted mean. Alternatively, to obtain the same result,

$$\frac{71}{\sqrt{4}} = \pm 36\%$$

In other words, it appears possible, on the basis of four visits, to count profundal sediment bacteria to within $\pm 36\%$, of which about one-fifth is due to handling errors.

In practice we have averaged the logs of the trip means instead of the means themselves, then reconverted to obtain the numbers quoted in Tables 3 and 4. Table 5 is an example of the method, which has the effect of lowering the mean by giving more weight to low-count trips than to high-count ones. Its basis of thought is that counts less than zero cannot occur to influence results, but occasional very high ones can, because of a bloom or some local situation. Table 5 has an example of an individual high count. The use of logs within each trip has not been resorted to because the variability within trips is not so great as between trips.

We do not know the cause of fluctuation between visits. Weeks (1944) found that in Western Lake Erie surface sediment aerobes showed increases following periods of phytoplankton maxima and those periods of high turbidity of lake water that were caused by river discharge. The numbers of anaerobic bacteria showed only slight change. As for ourselves, not having followed events from day to day we cannot say whether plankton blooms may have preceded the maximal values. While Millipore counts were restricted to the summer months, agar counts were made over the whole year, and they indicate that maxima were not seasonal. Thus on five lakes the times of highest and lowest counts are as follows. Where two or three dates are given, it means that practically identical values were recorded.

TABLE 4. *Bacteria in sediment and characteristics of lakes surveyed by others*

References for the final lake, Lauzon, are Kleerekoper and Grenier 1952, and Cooper *et al.* 1953. For other lakes, bacterial counts are from Henrici and McCoy 1938. Only counts to 5 cm mud depth are used. All counts were of colonies grown on agar. The same method of averaging counts and field trips has been used as illustrated for our own Montague Pond, Table 5. Lake characteristics from one or more of the following: Birge and Juday 1911, 1927, 1934; Black 1929; Hardman and Henrici 1939; Henrici 1939; Juday and Birge 1932, 1933, 1941; Juday, Birge, and Meloche 1935.

We are indebted to Dr. M. C. Sparr for sending water samples from Lakes Brazelle, Boulder, and Mendota which were analyzed to complete Table 4. Dr. S. Eddy kindly supplied information on Lake Alexander from his own survey.

Name	Location	Area ha	Mean depth m	Strati- fied	Color Pt-Co	pH	M.O. alk. as CaCO ₃ p.p.m.	Conductivity at 20° mega- mhos	Microorganisms		
									No. trips	No. stations	Thous. of col- onies per ml fresh sediment
Allen	Northeastern Wisconsin	16	0.9	No	277	8.8	31.8	62	2	2	87
Boulder		248	4.3		62	7.5	30.9	52	1	1	99
Crystal		30	9.7	Yes	2	6.1	3.1	10	1	1	5.1
Helmet		3	4.1		243	5.7	5.0	20	3	3	120
Little John		67	3.8		17	7.5	40.4	69	2	2	92
Mary		1	7.8	Yes	120	6.0	6.0	21	2	2	67
Muskellunge		372	7.0	Yes	5	7.3	22.3	42	3	3	19
Trout		1556	14.0	Yes	5	7.6	43.0	77	4	4	59
Weber		16	7.2	Yes	1	6.2	5.2	9	2	2	5.1
Mendota		3939	12.1	Yes	11	8.5	166.4	294	9	9	962
Alexander	Central Minne- sota	1130	9.0	No			91.3		6	7	108
Lauzon	Montcalm Co. Prov. Quebec	11	10.2	Yes	9	6.8	4.7		9	14	24

Lake	Maximum count	Minimum count
Bluff	July 16 Sept. 18	Aug. 2 June 9
Copper	Oct. 27 Dec. 30	Feb. 13
Grand	Aug. 29	July 24 Sept. 4
Lily	Aug. 22	July 2
Punchbowl	June 23 Oct. 12 Jan. 17	July 18

There is no obvious pattern in the dates given, and the conclusion is that for the present the fluctuations in bacterial population with time should be regarded as random and without known cause.

EFFECT OF SEDIMENT DEPTH

It is known that bacterial numbers decline with depth in marine sediments (ZoBell 1946) as well as in lakes (Henrici and McCoy 1938). The last mentioned authors draw attention to the resemblance between the apparently logarithmic decline with depth,

hence with time, to the survivorship curves of disinfection experiments. The sediment, according to this idea, is becoming disinfected with the passage of time. Long survival of bacteria is not unlikely, for they are reported in still wholesome canned foods after 113 years (Anon. 1954). Perfiliev (1929) found them in lake sediment at 1.6 m, age being estimated at over 1000 years, while Waksman and Purvis (1932) found bacteria and Actinomycetes to the same depth in a Florida bog. Bacteria are present down to 4 m in the sediment of Lake Biserovo, USSR, and exhibit an apparent logarithmic decline from the surface (Kuznetsov 1958).

We have examined deep cores on two lakes, one of them primitive and the other moderately acid bog. The cores, kindly taken by Dr. D. A. Livingstone with a sampler of his own construction (1955), went through several meters to base gravel. Bacterial counts are shown in Figure 4. There is a decline, which appears to be logarithmic, although the lower values are too close to

TABLE 5. *Method of calculating average bacterial counts*

The example, Montague Pond, Prince Edward Island, has fewer data than any other lake listed in Table 3. In the first visit Naumann cores were sliced; in the second visit small tubes were plunged into the surface of dredge samples. Each entry in column 3 is the mean of four petri dish counts. These are combined to give the arithmetic trip mean, of which logs are taken and averaged to give a log mean, and eventually in the bottom line, a mean count to be quoted. The effect of using logs is to suppress high counts and favor low ones. The per cent reduction by reason of using logs is

$$100 - \frac{433 \times 100}{554} = 21.8\%$$

1	2	3	4	5
Lake 15, Montague Pond, P.E.I.	Depth from surface cm	Arith. means of quadruplicate counts per ml fresh sediment Thousands	Trip value. Arith. mean of column 3	Log of column 4
First visit, July 14, 1954. One station occupied. Two Naumann cores taken and sectioned.	0.0-0.5	180	209	2.3201
	0.5-1.0	230		
	1.0-2.0	160		
	3.0-4.0	190		
	0.0-0.5	270		
	0.5-1.0	280		
	1.0-2.0	150		
Second visit, June 26, 1956. All surface cores in top 3 cm. Roman numerals in column 2 are stations I, II, etc. Letters are: <i>a</i> , incubated June 29; <i>b</i> , incubated July 10.	Station		898	2.9533
	I a	3469		
	I b	884		
	II a	529		
	II b	648		
	III a	271		
	III b	707		
	IV a	294		
	IV b	383		
Arithmetic mean			554	2.6367
Mean via logs, antilog 2.6367			433	

zero to prove it. Actually the values in deepest mud represent only about one colony on every second filter, which approaches the limits of measurement. No special interpretation can be given to the inflection in the Silver Lake graph at about 0.5 m, which is probably due to normal variability in the count.

The span of time represented by these cores may be taken as some 15,000 yr. According to Deevey (1955) the age of Linsley Pond is estimated from radio-carbon dating as 16,000 years. Tutin (1955) by count of annual layers and other data, gives 12,000 years for Lake Windermere. If our counts are valid they indicate therefore that micro-organisms can live for 15,000 years. Presumably they remain inactive, since degradation products of chlorophyll and free sugars are undestroyed in the sedimentary depths (Vallentyne 1957).

All the samples were counted both aerobically and anaerobically. There were no clear numerical differences between the methods, and they have been averaged to locate the points on the graphs. The anaerobic technique is theoretically a little safer, because contaminating bacteria seem to be obligatory aerobes. Contaminating colonies are also usually coloured yellow or pink, while the native ones are white to ivory. No distinction was made between bacteria and Actinomycetes. Molds did not appear other than as contaminants.

Changing the order of depth magnitude, we may now consider bacterial distribution in the top few cm. First of all, is there a very high concentration in the immediate surface layer? This would mean presumably a number beyond the logarithmic extrapolation from the deep mud. For marine muds Reuszer (1933) reports at the surface "a very thin layer containing organic matter in a less advanced stage of decomposition supporting a bacterial growth much richer not only in numbers but also in types of bacteria than the lower layers." Henrici and McCoy suggest the same for lakes, although in 16 out of their 39 cores the surface count was not the highest of the series.

We were expecting high surface counts, having seen a thin greenish layer, perhaps of sedimented algal cells, at the mud surface of cores taken in summer from several lakes. There was also the finding that radiophosphorus added to water of Jenkin cores exchanged with only a paper thin layer of sediment (Hayes 1955). Moreover, there is often distinguishable in a core, a light-weight, fuzzy, surface layer of low ash

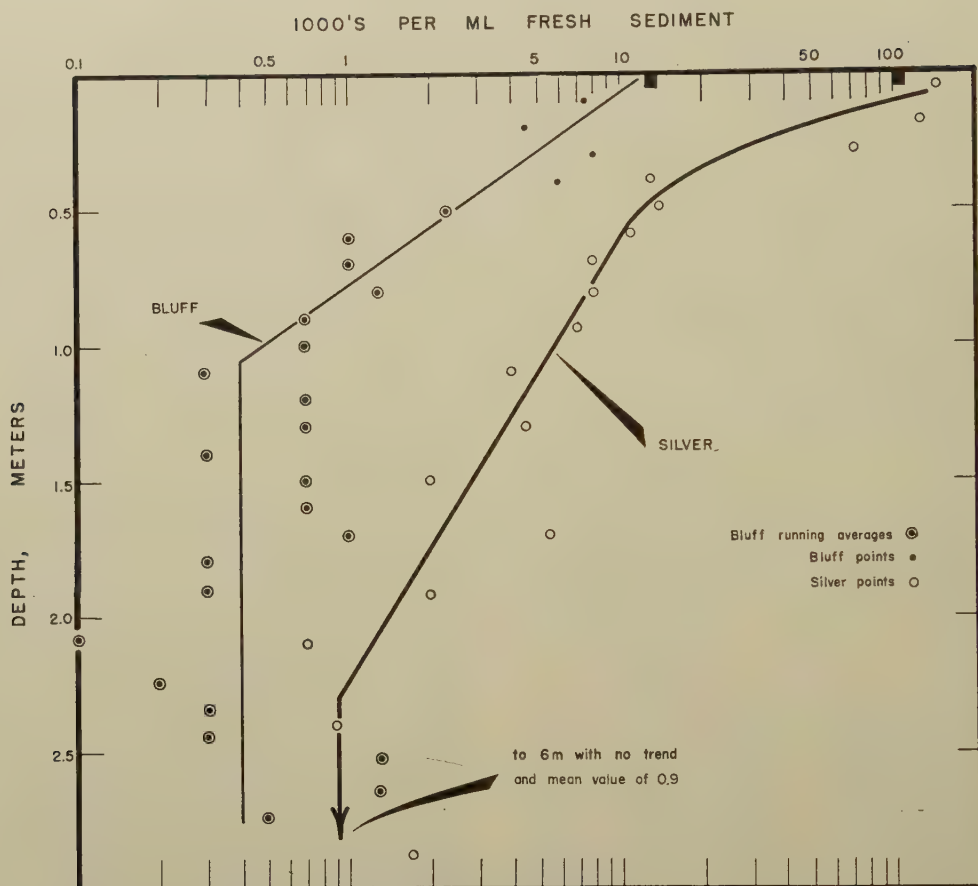


FIG. 4. Arithmetic-log plot showing bacteria in sediment cores of Bluff Lake (primitive) and Silver Lake (acid bog) based on anaerobic and aerobic counts. The sampler was driven to base gravel, which was reached at 2.75 m in Bluff and 6.0 m in Silver. Bottom half of Silver Lake curve not shown. The total core depth might correspond to 15,000 years, the time since the last ice age.

Rectangles at top represent average surface counts from Table 3 and show that the bog lake contains 10 times as many bacteria as the clear water one. The two lakes are within a mile of each other in the same granite-quartzite area.

For the Bluff Lake curve only one core was done. Many cultures below 0.5 m gave zero counts, which cannot be shown on a log scale. Hence a running average of three's is resorted to. The mean of the deeper counts is 0.4 which is so close to the blank that its validity is doubtful.

The Silver Lake graph is an average of two cores. There is a decline with depth to a value of 0.9 at approximately 2 m, below which there is no further trend. The deep counts, which went all the way to the bottom, were largely obtained from anaerobic cultures and may be genuine.

content, often several mm thick. However, the anticipations we had built up from all this evidence were not supported by the bacterial counts.

Some 40 Naumann cores from a dozen lakes in the Maritime Provinces have been sliced for bacterial counts. Usually the first cut is 0.5 cm down, the next at 1 cm, thence at 1-cm intervals down to a maximum of 15 cm. One object was to see whether

there was a strong concentration in the surface layer. It turned out that the ratio of counts of all top slices, mean depth 0.25 cm, to second slices (0.75 cm) was 1.28 on a dry weight basis and 1.07 on a fresh volume basis. The difference in the values is because the surface layer is flocculent. Probably the volumetric value is a better reflection of activity. The ratio 1.07 is the same as unity considering experimental errors; in

fact, if one high reading is omitted it becomes 1.017. The conclusion is that we cannot find a high-count surface layer; if it exists it must be extremely thin, something like 0.001 cm.

Counts and comparisons like those described have been made on sections of the cores down to 5 or 6 cm below the surface. Here again there is no trend detectable, and hence no demonstrable decline in bacteria to this depth. As for greater depth, 16 cores from 8 lakes were 10 cm or longer. When the mean of the two top counts is expressed as a ratio to the lowermost count (av. 12 cm down) the surface shows 6.48 times as many bacteria as the deeper slice. Thus it appears that the "surface," as far as bacteria are concerned, extends down for 5 cm approximately, below which a decline sets in, very likely a logarithmic one. Most of our lakes being shallow, it might be supposed that plant roots and bottom organisms are stirring up the mud. However even in deep lakes (Grand, Copper, Black Brook) where cores came from well below the plant zone, results were the same. In these lakes, of course, bottom fauna would be expected.

The mean ratios quoted were obtained by averaging the logs of the individual ratios, then reconverting each to a real number. This gives equal weight in the mean value to low and high ratios; for example the average of ratios of 10 and 0.1 would be 1.0, not 5.

Henrici and McCoy (1938) observe that some of their findings on depth-distribution relations support Naumann's (1930) proposed division into an upper or "actual" sediment layer, inhabited by burrowing bottom organisms, and a lower or "historical" layer. Naumann set the break at about 10 cm; Henrici and McCoy suggest 8-12 cm; our own results would indicate 5-10 cm depth. Only in exceptional cases is the "break" a sudden one; probably "gradient" would be a better designation.

SURFACE SEDIMENT BACTERIA IN RELATION TO PRODUCTIVITY

The counts in our lake sediments are given in Table 3, expressed on a volumetric

and on a dry weight basis. Counts on additional lakes, most of them in northeastern Wisconsin, are given in Table 4. As these latter are presented only on a volumetric basis, comparisons will be restricted to this unit. An example of the method of averaging results is given in Table 5.

Information on the Eastern Canadian lakes is given in the first paper of this series. The following notes will assist in characterizing the lakes in Table 4.

Allen or Brazelle, more recently called Brazelt, is very dark brown, turbid and weedy. The water apparently comes from sphagnum bogs. It is stated to be a better producer (of plankton) than Helmet, which it resembles in water color, but which it greatly exceeds in alkalinity.

Boulder was visited only once for bacterial sampling. The water which enters it is dark brown from bog drainage, but the effluent water is quite clear.

Crystal also was sampled only once. The water is very clear and mineral content very low: "quite the lowest in productivity of any lake we have studied" (Henrici and McCoy 1938).

Helmet is called a dystrophic lakelet by Juday and Birge (1933) who discuss its color and light penetration on pp. 234-5.

Little John is called eutrophic.

Mary has more than 18 mg organic carbon per litre and is described as the extreme example of a lakelet receiving carbon from outside sources.

Muskellunge or Big Muskellunge is a seepage lake with no permanent outlet.

Trout is the largest and deepest of the N. E. Wisconsin group. It is nearly divided into two lakes. A report by Carpenter (1941) states that bacteria vary from 4.7 to 250 thousand per g wet surface mud. There are no tables of counts, but the mean of the two values via logs is 37, which is surprisingly close to the number 59 in Table 4.

Weber is described as similar to Crystal Lake but more productive because of fertilizing experiments. "Various fertilizers including lime have been added during the past few years" (Allgeier *et al.* 1941).

Mendota is too well known to require comment. Only the profundal stations sampled by Henrici and McCoy have been

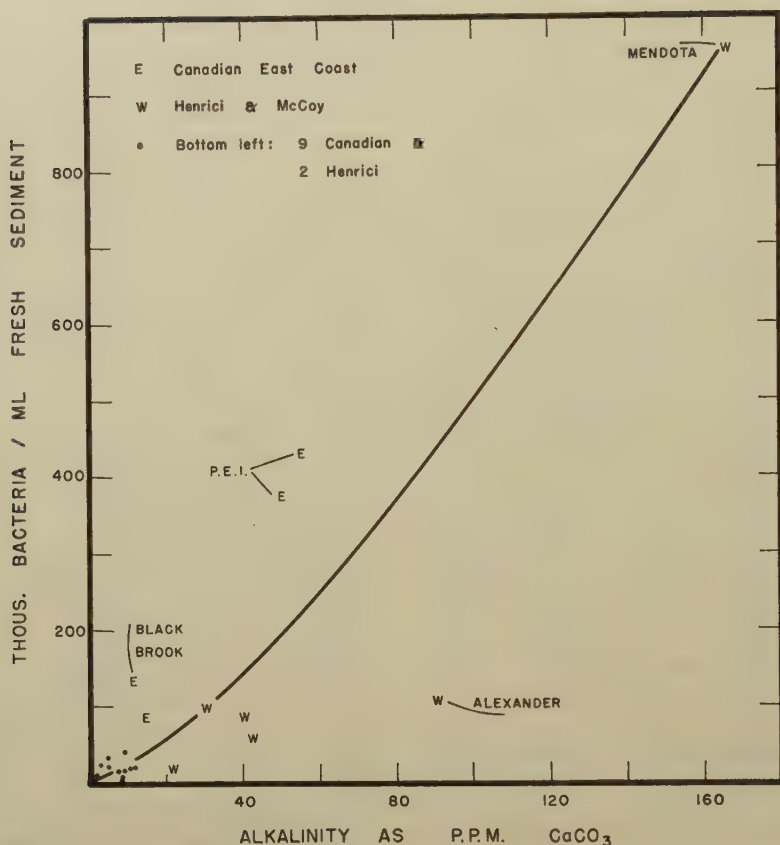


FIG. 5. Relation of carbonate, measured as methyl orange alkalinity, to bacterial counts. Six lakes whose color is over 100 are omitted, although one of them, Allen, with considerable alkalinity would fall on the line as drawn. Data for this and the following figures are taken from paper I, Table 1, and Tables 3 and 4 of this paper.

averaged to secure the quoted count; littoral stations are omitted.

Alexander drained into the Mississippi until about 1928, after which it became a seepage lake. Lime-loving plants, especially *Chara*, are reported to be abundant in some areas. The lake provides excellent sport fishing. Two littoral stations of Henrici and McCoy are omitted from the figures averaged to get the value quoted.

Lauzon in the Laurentian Shield is a primitive lake like our Bluff. Its bacterial mud counts fluctuated violently, unparallelled by any other lake under consideration. The ratio of maximum station count to minimum was 2×10^6 . Some of the difficulty was due to media. Restricting the results to the best medium tried (similar to ours, less vitamins and caseinate) and to one

summer (1950) and to trip averages, the ratio of maximum to minimum was 345, still very high. Using Table VII values (Cooper *et al.* 1953) which, with one exception, were non-peak days, the high-to-low trip ratio is 17, which is not excessive. The Lauzon maxima occurred in three peaks, each apparently due to an outburst of a single organism, each time a different one. The high counts occurred all over the lake and were independent of the character of the sediment. The authors' Table VI gives counts on the non-dominant types, which are perhaps more nearly comparable to other lake results and have been used in our Table 4. The ratio of maximum on a trip to minimum is down to 6.8, a quite ordinary value by comparison with results elsewhere.

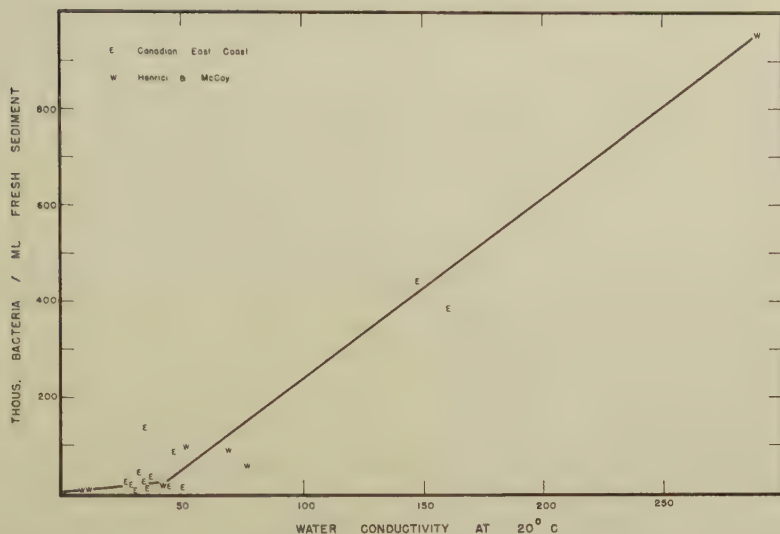


FIG. 6. Relation of bacterial counts to water conductivity. As in the previous illustration, highly colored lakes are omitted.

Lauzon data have not been used in constructing graphs for this paper.

ALKALINITY

The alkalinity of lakes, measured as calcium or magnesium or carbonate, is one of what might be called the colligative properties of productivity. The carbonate titration values are plotted against bacteria in Figure 5. The upward sweep is fairly clear, although one would wish for more points in the middle part. On this showing bacteria have some value as an index of productivity.

CONDUCTIVITY

Outside of certain special effects (see *e.g.* paper I), lake conductivity is due to calcium and magnesium salts. It is not surprising therefore that bacteria should vary directly with conductivity as shown in Figure 6. The curve appears to flatten at the lower left corner, since even low conductivity water still has some bacteria. Possibly if more data were at hand a semi-logarithmic plot would yield a single straight line.

THE BOG CORRECTION

It is generally known that brown lakes, although reckoned as unproductive, nevertheless have a rapid oxygen depletion in the

depths. One might expect a high bacterial count in the sediments as well. The relation is complicated by the fact that high calcium content, or methyl orange alkalinity, is also accompanied by a high bacterial count. To screen out the stimulus of alkalinity and examine the relation of bacterial numbers to water color alone, we have plotted in Figure 7 only those lakes whose M.O. alkalinity was below 10. The relationship is quite clear, and provides a basis for stating what part of the bacterial count of any lake is to be attributed to bog. When more lakes are added to our oxygen series (paper V) it should be possible to assign similarly a known fraction of the oxygen consumption to a bog effect, as evidenced by color. Subtraction of this from the total will leave a real or useful bacterial or oxygen value which is related to productivity.

ABERRANT LAKES

There are 3 lakes which have too few bacteria, as judged by their positions in one or more of Figures 5, 6, and 7, and general expectations. One is no. 12, Crecy Lake, which has been fertilized several times. A large growth of plants over the bottom made it difficult to take dredge and core samples. Another is no. 4, Boar's Back, whose highly colored water would lead one to anticipate a

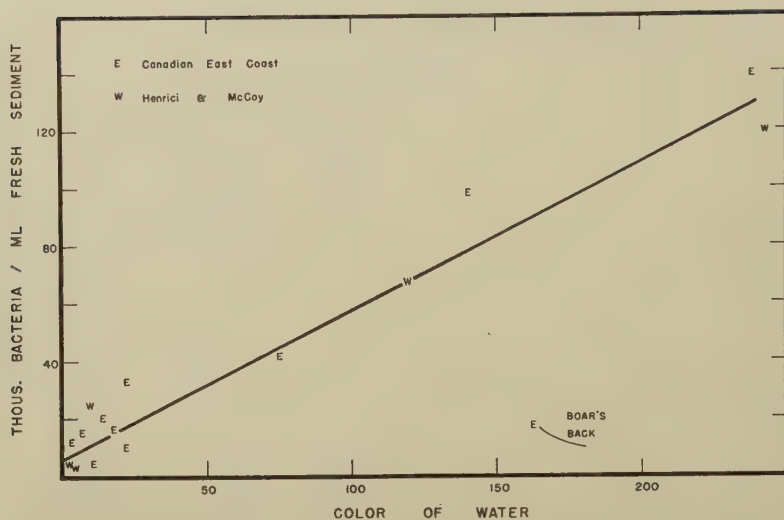


FIG. 7. Relation of bacterial numbers to water color. Only lakes with methyl orange alkalinity less than 10 are included. Color in Pt-cobalt units from paper I. The lake which is far below the line, with a color value of 163, is Boar's Back.

large standing crop of bacteria. Here, too, much of the bottom is covered with rooted aquatics making it difficult to use a dredge. The third is Lake Alexander, Minn., about which we have less chemical information than any other. This is also an unstratified lake, much of which consists of shallow bays with very abundant plant life.

We suggest the possibility that competition may exist between plants and bacteria, so that the former perhaps hold nutrients from the open water and reduce the fall-out to the profundal zone. (Bacteria do flourish in the littoral zones and shallow bays of Lake Alexander, which are not considered in our averages.) Alternatively there may be a specific antibiotic effect.

On the other side, Black Brook Lake has more bacteria than its chemical properties would suggest. It is also a high producer of trout which however remain stunted. Further study will be needed here.

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The Physical Oceanography of Narragansett Bay¹

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ABSTRACT

Analyses of pertinent data from various physical oceanographic programs carried out in Narragansett Bay are presented in an effort to give a useful description of tidal characteristics, temperature and salinity distributions, and the nontidal circulation. The observed tides and tidal currents are shown to be the result of a standing tidal wave with a slight progressive component and a damping coefficient of 1.6. Both temperature and salinity distributions indicate essentially homogeneous bottom water in the upper part of the Bay as contrasted with the surface layer which possesses marked progressive changes over the same area. One direct measurement and the salinity distribution seem to confirm the nontidal circulation calculations which are based on river run-off, surface nontidal currents, and cross sectional areas. Narragansett Bay appears to be an estuary in which the salt balance is maintained, under steady state conditions, by horizontal advection, vertical advection, and vertical eddy diffusion.

INTRODUCTION

Narragansett Bay (Fig. 1) is composed of three north-south interconnected passages: a) West Passage, b) East Passage and the Providence River, and c) Mt. Hope Bay and the Sakonnet River. The latter body of water, though termed "river," is more properly classed as a passage.² The Bay is fed, principally, by the Providence River and its tributaries, the Taunton River which flows into Mt. Hope Bay, the Potowomut River, and the Warren-Barrington River. Narragansett Bay is exposed to the open ocean via Rhode Island Sound to the south by entrances to West and East Passages and the Sakonnet River.

The area of the Bay at mean low water is 94.5 naut. mi.² The total volume below mean low water is 10^{10} ft³. The area at any given depth and the volume below any given depth are given in Figure 2. The mean depth of the entire Bay is 29 ft, and the maximum (350 yards North of Castle Hill Light) is 188 ft below mean low water. Of particular significance is the contrast between the mean depth of lower East

Passage which is 58 ft and West Passage and the Sakonnet River which are both 24.5.

Prior to 1951, physical oceanographic studies were confined to tides and tidal currents. The latter, based on observations extending from 1844 (by G. S. Blake) through 1931 (mostly by J. C. Sammons in 1930), have been analyzed and published by Haight (1938). Also, float experiments in the upper reaches of the Bay were conducted by Gray (1884). Since 1951, extensive physical oceanographic programs have been carried out by the Narragansett Marine Laboratory of the University of Rhode Island with financial assistance at various times from the U. S. Navy Hydrographic Office [Nonr-396(01)], Office of Naval Research [Nonr-396(05)], and the Corps of Engineers, U. S. Army (DA-19-016-CIVENG-56-1071). These studies have been primarily concerned with bottom currents and the distributions of temperature and salinity in both Narragansett Bay and Rhode Island Sound.

The temperature and salinity sections of the present paper are based on the data from nine cruises listed in Table 1. A total of 409 hydrographic stations were made in 129 station locations in the Bay. On Cruises 2, 4, 5, and 7, stations were made at both slack before flood and slack before

¹ Contribution No. 21 from the Narragansett Marine Laboratory of the University of Rhode Island.

² It is interesting to note that on Blaskowitz's (1777) chart the Sakonnet River is termed the Seakonnet Passage.

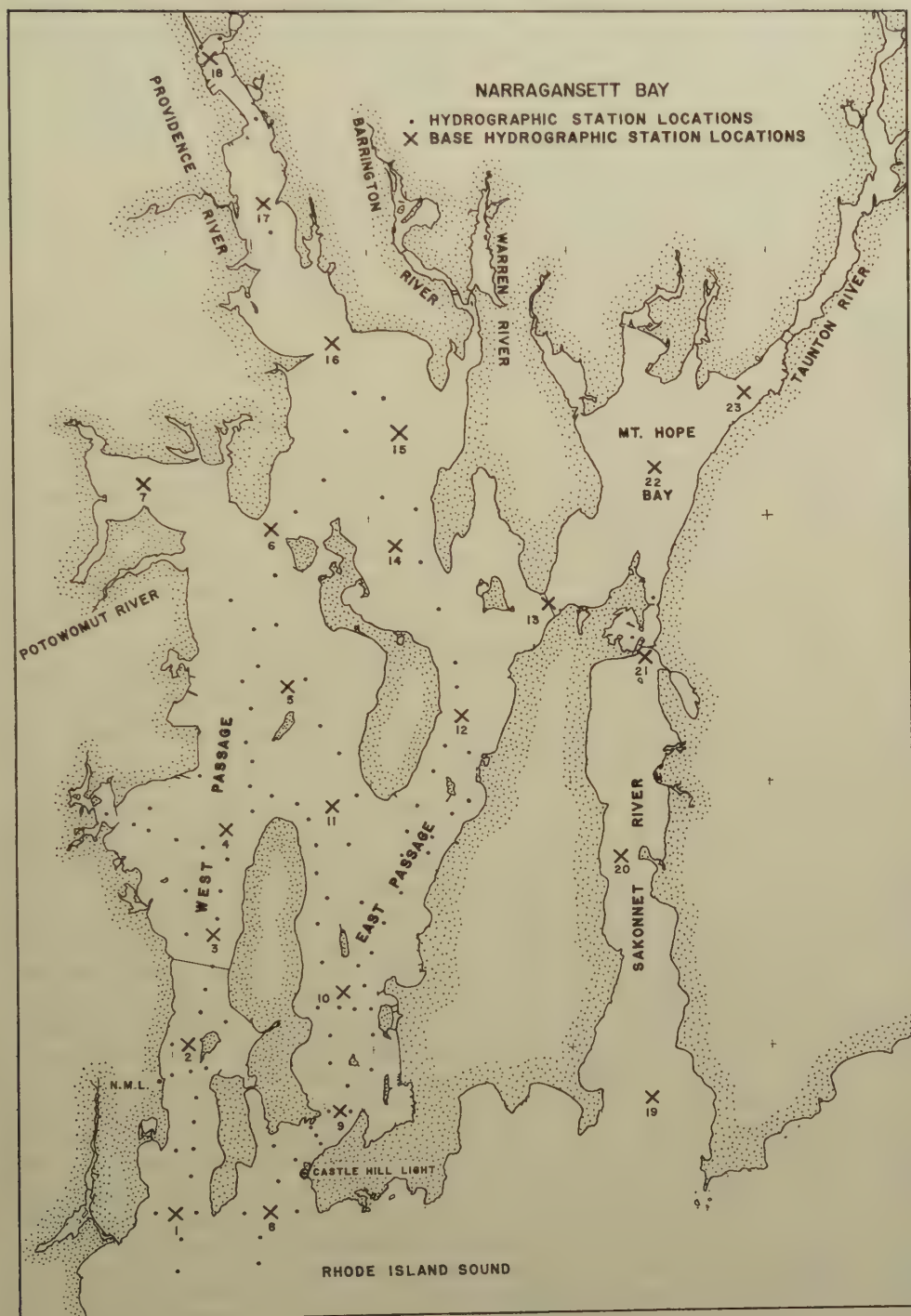


FIG. 1. Station locations (See text for explanation).

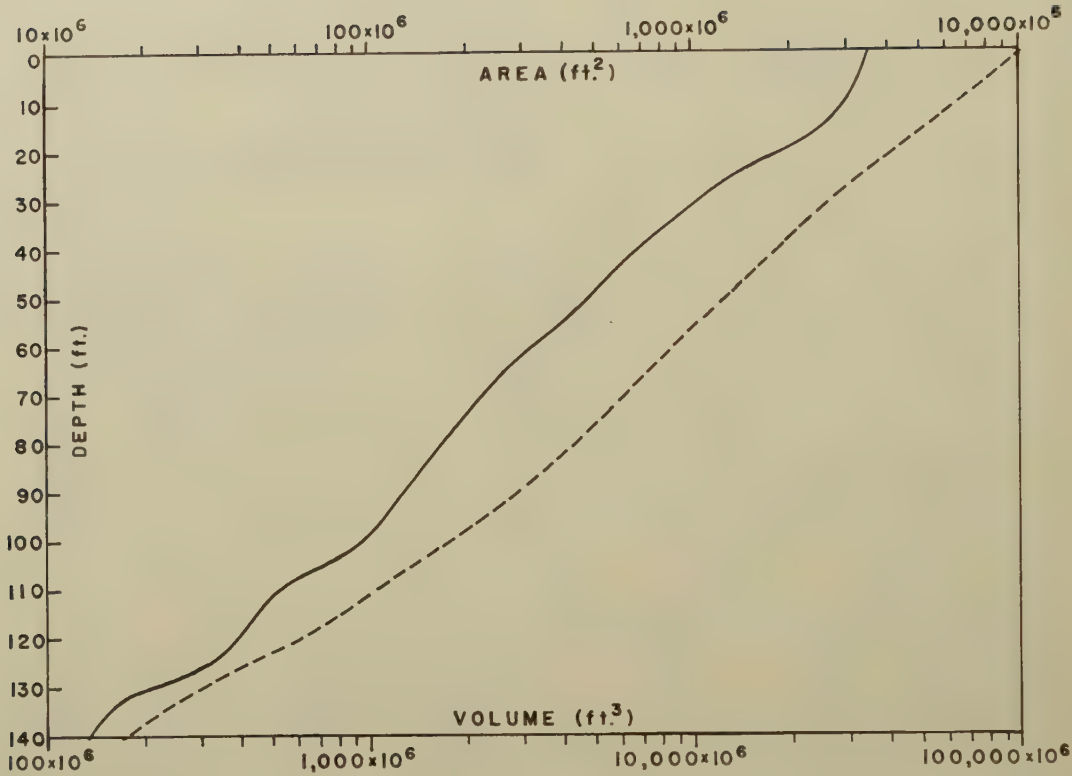


FIG. 2. Area of Narragansett Bay at any given depth (solid line) and volume below any given depth (dashed line). Depths corrected to mean low water.

TABLE 1. *Hydrographic cruises in Narragansett Bay*

Cruise number	Dates	Number of stations made	Number of station locations
2	Aug. 24 to Sept. 18, 1951	176	88
4	Feb. 19 to 22, 1952	50	25
5	Mar. 25 to 27, 1952	50	25
7	Aug. 13 to Sept. 16, 1952	90	45
14	Feb. 1 to 21, 1956	23	23
15	Apr. 2 to 20, 1956	23	23
18	June 11 to 28, 1956	23	23
19	Aug. 6 to 10, 1956	23	23
Series at Sta. 4	Nov. 4, 1954 to Aug. 31, 1955	51	1

ebb. On Cruises 14, 15, 18, and 19, stations were made at slack before ebb only. For convenience, the sequence of periodic observations at a single location is designated as a series followed by the station number. Station locations are shown in Figure 1. Those indicated as base hydro-

graphic station locations were chosen as the network for the temperature and salinity analyses since they were occupied on the most synoptic cruise (Cruise 19) and were also the most frequently occupied since 1951. Temperatures were taken with protected reversing thermometers and bathythermograph, and water samples were obtained with Nansen bottles and titrated for chlorinity by the Knudsen method using standard water. The following persons assisted in the collection and processing of the data: Stanley T. Spink, Robert Campbell, Theodore J. Wehe, David E. Frazier, Kenneth G. Gallup, and Charles V. Mulholland. Acknowledgement is also made to Dr. Bostwick H. Ketchum for his helpful suggestions.

TIDES

The tidal wave in Narragansett Bay is semidiurnal and possesses essentially standing wave characteristics. The mean range

increases from 3.5 ft at the entrances to 4.6 ft at the head (4.4 ft to 5.7 ft at spring) (U.S.C. & G.S., 1958b). There is a progressive component, however, which causes a stage lag of 10 minutes from the entrances to about the latitude of Wickford (41° 35' N). If we assume that the observed tidal wave is composed of two superimposed damped progressive waves, one moving up the Bay and the other, its reflection, moving out of the Bay, then the observed wave may be expressed according to Redfield (1950) by the equation

$$\eta = A[\cos(\sigma t - kx)e^{-\mu x} + \cos(\sigma t + kx)e^{\mu x}]$$

where η is the elevation, A the amplitude at the head, σ the change in phase per unit of time, k the change in phase per unit of distance, t the time measured from the time of high water at the head, x the distance measured from the head, and μ the damping coefficient. Using Redfield's method, which utilizes the mean tidal range and high-water intervals, a damping coefficient of 1.6 was found. This contrasts with 1.0 which Redfield determined for Long Island Sound and the Bay of Fundy. It was to be expected that a damping coefficient as large as 1.6 would be found for Narragansett Bay, owing to its many channel restrictions and irregularities.

For verification the actual and theoretical time differences between high and slack water are compared using the coefficient previously found. No verification was found for Narragansett Bay by using the data from the Current Tables (U.S.C. & G.S., 1958a), which Redfield used for Long Island Sound and the Bay of Fundy, or the more detailed surface and bottom current data from Haight (1938). Verification was found, however, by utilizing data from seven long-period stations, conducted by the Narragansett Marine Laboratory, at which accurate times of slack water were obtained near the bottom. This verification was probably possible because the near bottom observational level reduced direct river discharge and meteorological effects, and also because the longer periods of station occupation made possible the more accurate determinations of slack water.

Tidal currents also exhibit the characteristics of a standing type of tidal wave. Strengths occur approximately midway between high and low water, and slacks coincide approximately with high and low water (U.S.C. & G.S., 1958a, b). Generally, the strengths amount to about $\frac{1}{2}$ to 1 knot, although a maximum of $2\frac{3}{4}$ knots occurs at the Sakonnet highway bridge (Haight 1938). As one proceeds from the mouth to the head, a double strength of flood becomes more pronounced. Haight has shown that this is accounted for by the natural period of oscillation of the Bay lying between the periods of the M^4 and M^6 constituents. Haight also shows that the observed currents in Narragansett Bay can be represented, to a high degree of accuracy, by the combination of the M^2 , M^4 , and M^6 constituent tides. A marked exception to the above generalizations occurs at the Sakonnet River bridges where the current stages are from 1 to $1\frac{1}{2}$ hours earlier.

TEMPERATURE DISTRIBUTION

Figure 3 shows the horizontal temperature distribution at slack before ebb ± 1 hour in August 1956 (Cruise 19, the most synoptic and extensive cruise). It illustrates late summer conditions of maximum yearly temperatures and minimum yearly run-off. Of particular note are the surface temperatures of the Sakonnet River which were about 4.5°F warmer than East Passage, and those of West Passage which were only about 0.9°F warmer than East. Also, from the middle of the Bay northward, the bottom temperatures were essentially uniform at about 69°F, while the surface temperatures increased from 68 to 73.5°F over the same distance.

The seasonal cycle throughout the Bay in 1956 is illustrated in Figures 4 and 5. The greatest increase in temperature occurred between April and June. With minor local exceptions, the temperature generally increased from the entrance to the head. A major exception occurred in the Providence River and East Passage in February when the surface water temperature was constant from the entrance to the head of the Bay. Another major exception

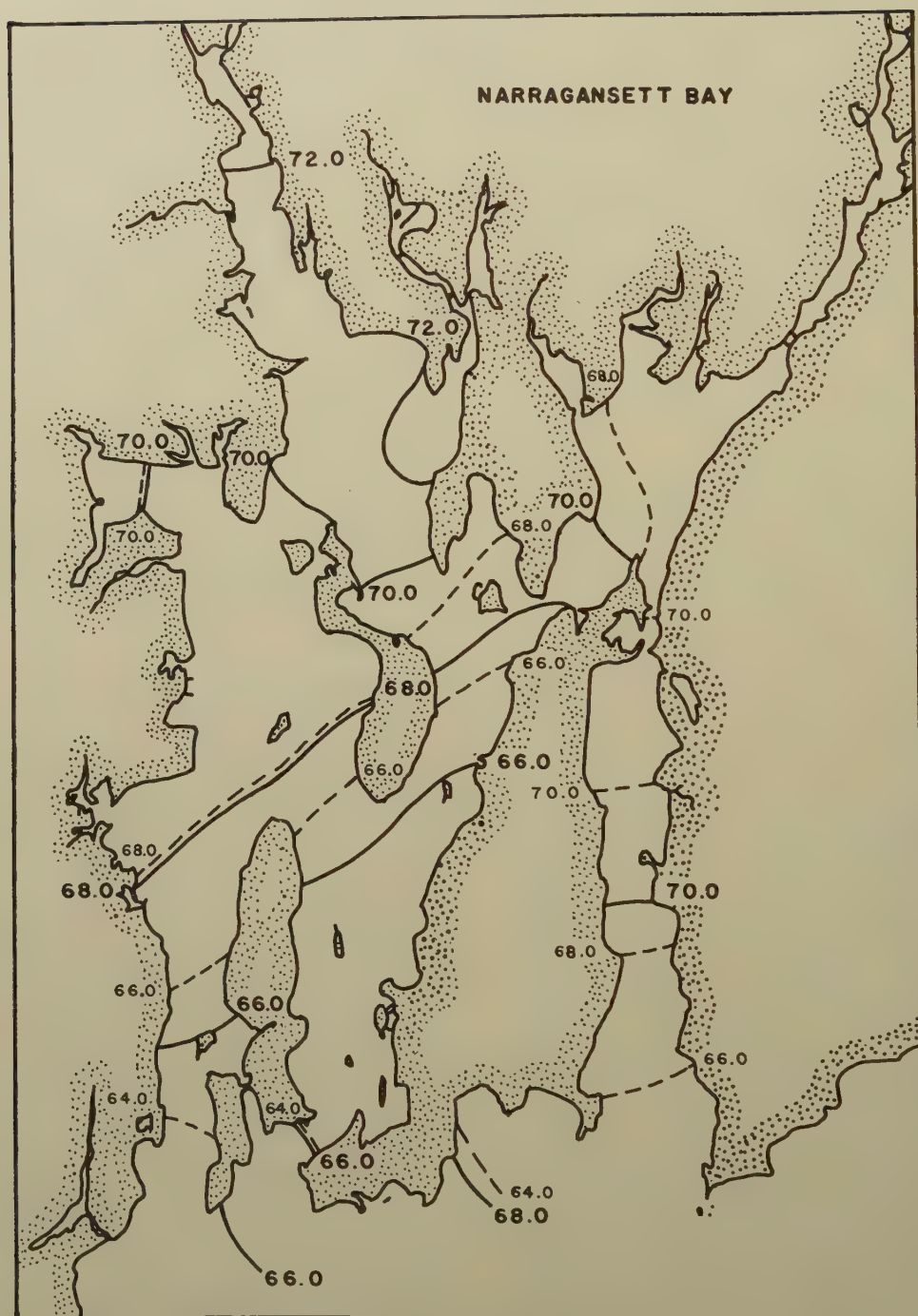


FIG. 3. Horizontal temperature distribution at slack before ebb ± 1 hour in $^{\circ}\text{F}$. Cruise 19. Surface—solid lines and large numerals. Bottom—dashed lines and small numerals.

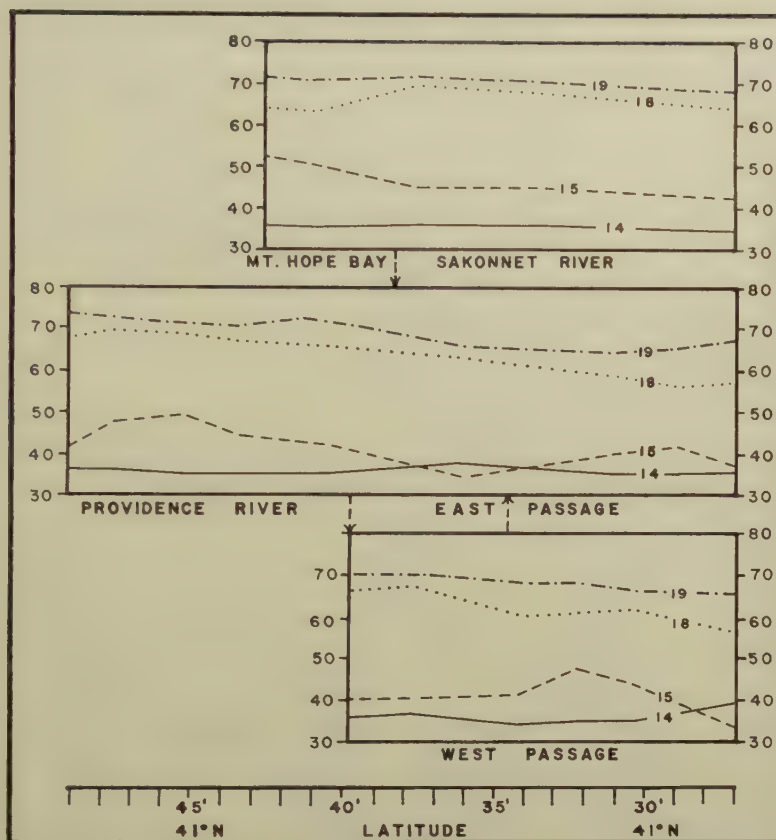


FIG. 4. Surface temperature distribution at slack before ebb ± 1 hour in $^{\circ}\text{F}$ along axes of channels during February (14), April (15), June (18) and August (19), 1956. Vertical dashed arrows indicate interconnections between passages and point in direction of net nontidal flow.

occurred in February in West Passage where the surface temperature tended to decrease toward the head. Essentially constant temperature bottom water was found to extend from the head to the entrance in the Providence River and East Passage in February. Major fluctuations were evident in April at both the surface and bottom in the Providence River and East Passage as well as in West Passage.

The vertical structure over a yearly cycle at Station 4 is shown in Figure 6. This figure was constructed from bathythermograms obtained from November 1954 through October 1955. Surface maxima of greater than 74°F were observed in early July and again in August, the latter extending to the bottom. A minimum of less than 35°F appeared in early February.

Maximum spring warming occurred in May. This agrees with the maximum warming in 1956 as indicated in Figures 4 and 5. Although the water was approximately isothermal from the surface to the bottom throughout most of the year, intense stratification was recorded in May (11°F decrease from surface to bottom) and again in early July (10°F decrease). The latter corresponds with the July surface maximum. Slight increases in temperature with depth were found in November and January.

An estimate of the variation from year to year is obtained by comparing the surface and bottom temperatures from Cruises 2 (Aug.-Sept. 1951), 7 (Aug.-Sept. 1952), and 19 (Aug. 1956). They show, on the average, that in the late summer of 1956

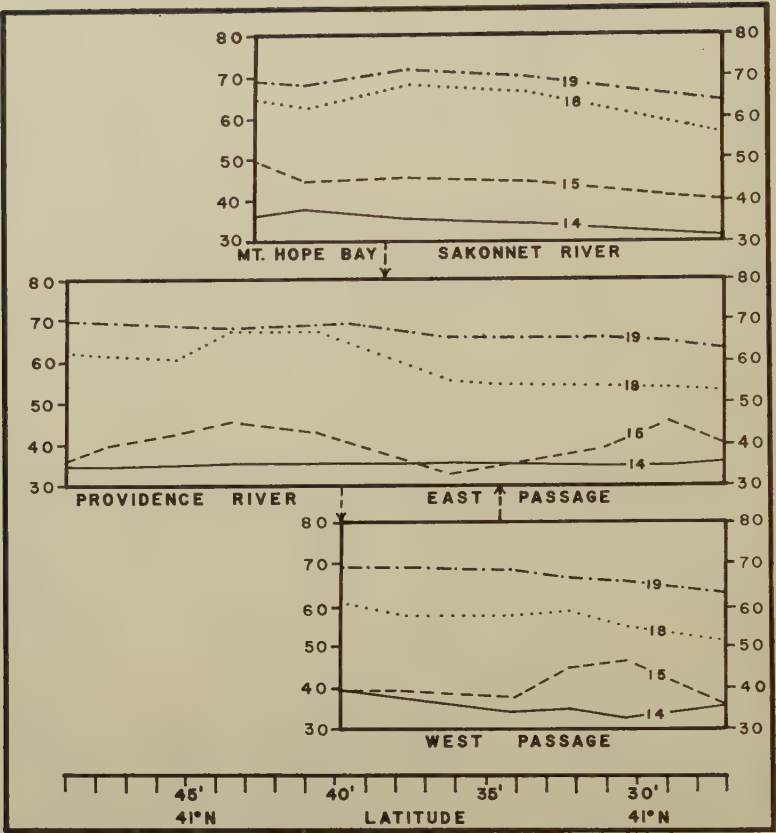


FIG. 5. Bottom temperature distribution at slack before ebb ± 1 hour in $^{\circ}\text{F}$ along axes of channels during February (14), April (15), June (18) and August (19), 1956. Vertical dashed arrows indicate interconnections between passages and point in direction of net nontidal flow.

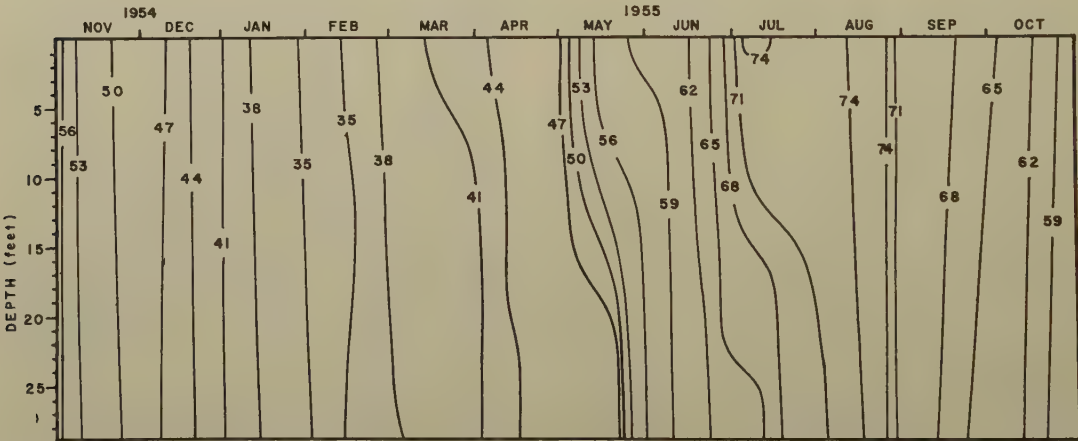


FIG. 6. Vertical distribution of temperature in $^{\circ}\text{F}$ at Station 4, November 1954–October 1955.

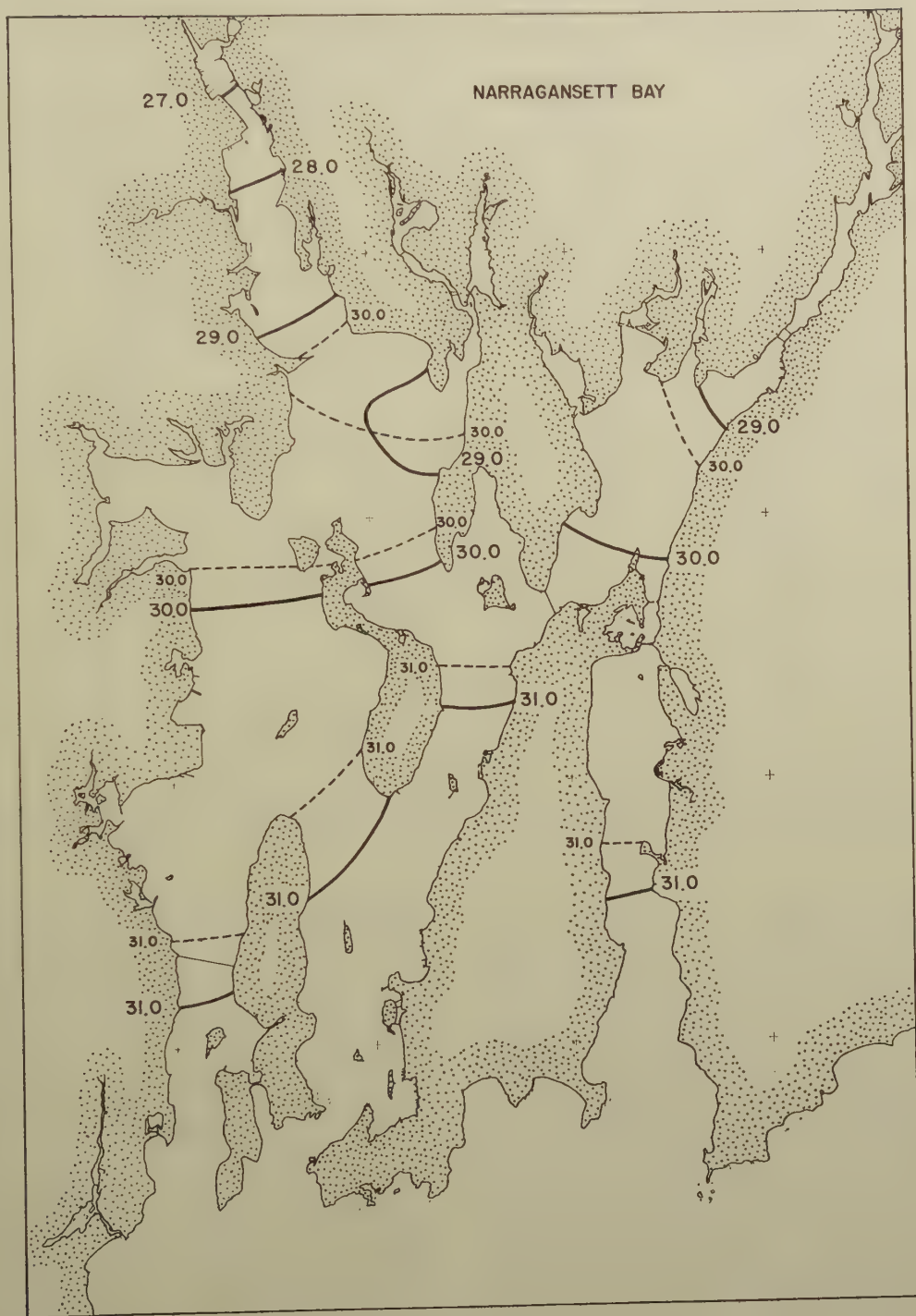


FIG. 7. Horizontal salinity distribution at slack before ebb ± 1 hour in ‰. Cruise 19. Surface—solid lines and large numerals. Bottom—dashed lines and small numerals.

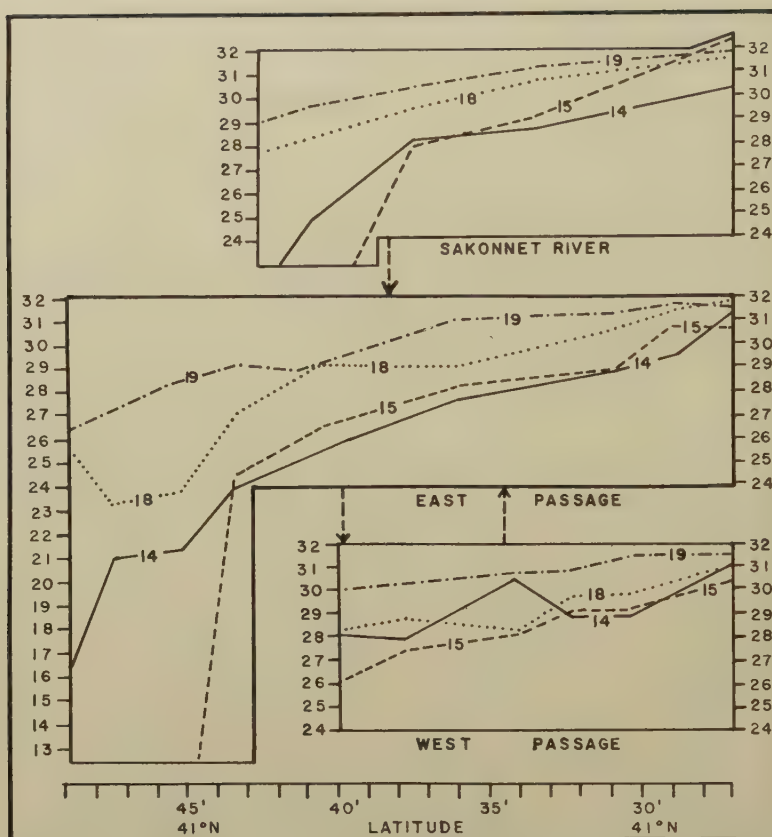


FIG. 8. Surface salinity distribution at slack before ebb ± 1 hour in ‰ along axes of channels during February (14), April (15), June (18) and August (19), 1956. Vertical dashed arrows indicate interconnections between passages and point in direction of net nontidal flow.

the water was 2.0°F cooler than in 1951 at the surface and 1.3°F cooler than in 1951 at the bottom. The temperature was 4.7°F cooler in 1956 than in 1952 at the surface but was 0.9°F warmer than in 1952 at the bottom. The data also show that the temperature differences between years (late summer) decreased from the entrance to the head. This decrease amounted to about 4.0°F at the entrances to West and East Passages and about 0.7°F at the mouth of the Providence River. It would thus appear that temperature variations from year to year are due to changes in the coastal waters and/or direct meteorological effects rather than to the indirect river contribution effect.

SALINITY DISTRIBUTION

The distribution of salinity in Narragansett Bay is illustrated in Figures 7, 8, and 9.

The salinity increased from the head to the entrances and from the surface to the bottom (Fig. 7). The bottom water was essentially uniform in salinity, as it was in temperature, from the middle of the Bay to the head, while the surface salinities progressively decreased over the same distance. Lower East Passage was markedly more saline than either West Passage or the Sakonnet River at both the surface and bottom.

Figures 8 and 9 illustrate the seasonal cycle along the axes of the three north-south passages. Generally the salinity increased from February to June, but there were major exceptions. The bottom water in West Passage may be considered as having had no marked seasonal variations (in comparison with the other passages), although its temperature, on the contrary (Fig. 5), did. Comparable conditions pre-

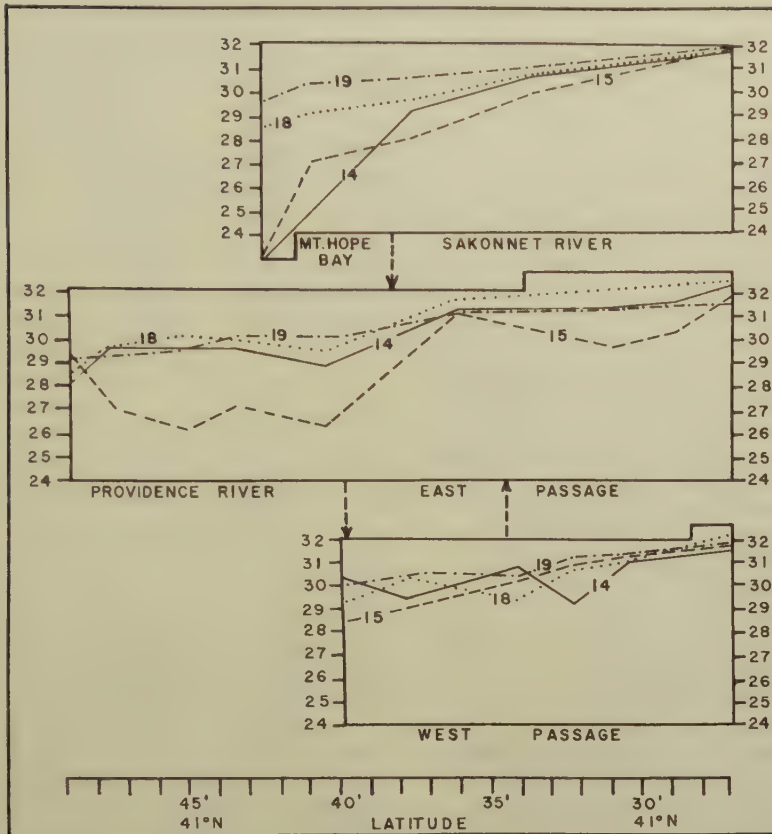


FIG. 9. Bottom salinity distribution at slack before ebb ± 1 hour in ‰ along axes of channels during February (14), April (15), June (18) and August (19), 1956. Vertical dashed arrows indicate interconnections between passages and point in direction of net nontidal flow.

vailed in the Providence River and East Passage except for the major fluctuations exhibited in the April curve (15). Extreme minima in surface salinity were found in April in both the Providence River and Mt. Hope Bay. In the Sakonnet River and East Passage, however, minimum salinities were generally found in February.

Salinity variations from year to year appear quite small in comparison with longitudinal or seasonal variations. A comparison of data from Cruises 2 (Aug.-Sept. 1951), 7 (Aug.-Sept. 1952), and 19 (Aug. 1956) indicates that, on the average, in the late summer of 1956 the water was 0.2‰ less saline than in 1951 at the surface and 0.3‰ less saline than in 1951 at the bottom. On the other hand, the 1956 salinities were, on the average, 0.7‰ greater than in 1952 at the surface and 0.2‰ greater than in 1952 at the bottom.

NONTIDAL CIRCULATION

Although numerous series of current observations have been conducted, lack of directional observations in all but three subsurface series have made subsurface nontidal reductions unreliable.³ A partially indirect determination of the nontidal circulation can be made, however, utilizing surface nontidal currents, distribution of salinity, river run-off, and cross sectional areas as suggested by Riley (1952).

The surface nontidal currents were taken from Haight (1938). Nine stations, occupied in July, August, and September of 1930, were selected for their appropriate location at ends and interconnections of

³ Unpublished subsurface observations by the U. S. Coast and Geodetic Survey in 1953 using Roberts Current Meters (recording both direction and velocity) were also inadequate for reduction due to the short lengths of the series.

TABLE 2. *Nontidal circulation in Narragansett Bay*

Locations	Sta. No.	River runoff cfs	Surface nontidal current ³ knots	Depth of no nontidal motion ⁴ ft	Area above level of no motion ⁵ ft ²	Area below level of no motion ⁵ ft ²	Surface layer		Bottom layer	
							Transport ⁶ ft ³ /sec.	Speed ⁷ knots	Transport ⁸ ft ³ /sec.	Speed ⁹ knots
Providence R.	16	334 ¹	.22	9	33600	55200	9374	.17	9040	.10
West Passage	4	88 ²	.08	11	107201	152269	10827	.06	10739	.04
	1	88	.24	30	196002	108802	59585	.18	59497	.32
East Passage	14	160	.05	15	120002	89601	7680	.04	7520	.05
	13	158	.05	27	49867	30667	3191	.04	3033	.06
	11	96	.11	6	52801	228536	7392	.08	7296	.02
	8	414	.37	66	360804	261337	169578	.28	169164	.38
Mt. Hope Bay	23	220 ¹	.03	6	9867	42933	385	.02	165	.00(2)
Sakonnet R.	19	62	.07	27	264003	115201	23496	.05	23434	.12

¹ From U.S.A.E. & U.S.G.S. for July 1930.

² 10 cfs added between Stations 6 and 5 from Potowomut R.

³ From Haight (1938).

⁴ Assumed at level of max. salinity gradient.

⁵ From U.S.C. & G.S. charts and depth of no nontidal motion.

⁶ Speed of surface layer times area above level of no motion.

⁷ $\frac{3}{4}$ of surface nontidal current.

⁸ Surface transport minus river water.

⁹ Bottom transport divided by area below level of no motion.

the various passages and for their coincidence with salinity stations of Cruise 19. In Figure 1 they are Stations 1, 4, 8, 11, 13, 14, 16, 19, and 23. Cruise 19 (August 1956) was selected for salinity comparison because of the very close agreement between the river run-off in the late summers of 1930 and 1956.⁴

The surface nontidal current velocity at the entrance to each passage (setting south in all passages) was multiplied by $\frac{3}{4}$ to give the average velocity (Sverdrup *et al.* 1942) and then multiplied by the cross sectional area to obtain the transport. The sum of the resultant transports for the entrances to the three passages was far greater than the river water contribution. Since this cannot happen without lowering the water level, it is evident that we have a typical two-layered estuary with nontidal flows out of the Bay at the surface and in at the bottom.

The level of no horizontal nontidal motion was assumed to occur at the level of the maximum salinity gradient. The average southward-setting surface nontidal trans-

ports were calculated by multiplying the nontidal velocities by $\frac{3}{4}$, to give the average velocities, and then multiplying by the cross sections area above the level of no nontidal motion. The average northward-setting bottom nontidal transports were calculated by subtracting the river water contribution from the value of the southward setting surface transport at the place in question.

The quantity of river water forking into the different passages is dependent on the relative size of the openings to the passages and on any channeling effects. This was determined collectively by multiplying the average ebb tidal flow by the cross sectional areas at the openings and then determining the percentage of flow to each. The channeling effect turned out, surprisingly, to be very small.

The results of these nontidal circulation calculations are given in Table 2. Verification of this study by direct measurements in the bottom northward moving current was possible in only one instance. This was a series of reduced tidal current measurements, with directions, conducted from August 1-12, 1889, by J. E. Pillsbury (Haight 1938) at a depth of 60 feet at Station 10 on Figure 1. (The assumed depth of no motion at this station is 45

⁴ River run-off data were provided by the Corps of Engineers, U. S. Army and U. S. Geological Survey. Bathymetric information was taken from standard U. S. Coast and Geodetic Survey charts.

feet and the depth of water, 80 feet.) Pillsbury's nontidal value was 0.13 knots as compared with 0.18 found in this study for the same location.

The nontidal circulation can also be determined from the salinity distribution in the Bay, the offshore salinity, river run-off, and cross sectional areas as described by Ketchum (1950). No offshore salinities were measured on Cruise 19. However, the offshore salinity necessary to mix with the Bay water to account for the circulation described above can be determined by working backward in Ketchum's method. The necessary offshore salinities were calculated in this manner and found to average 31.1‰. Such salinities were found southeast of the entrance to East Passage in Rhode Island Sound on cruises in the late summers of 1951 and 1952. The nontidal current in Rhode Island Sound south of Narragansett Bay sets west and then southwest hugging the Narragansett shoreline to Pt. Judith. Although not definitely confirming it, the latter considerations, together with the single direct measurement, indicate that our previous calculations are approximately correct. The distribution of salinity and calculated nontidal currents indicate that Narragansett Bay can be classified as a Pritchard (1955) Type B estuary (Type 2, Stommel and Farmer 1952). That is, under steady state conditions, the salt balance is maintained

by horizontal advection, vertical advection, and vertical eddy diffusion.

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Two Sources of Error in the Oxygen Light and Dark Bottle Method¹

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ABSTRACT

Rates of oxygen uptake per bacterial cell in dark bottles containing bacteria but no phytoplankton were applied to the bacterial population of the dark bottle in two-day light and dark bottle experiments and showed that, in the temperature range 11–21°C, bacteria were responsible for 42.5% to 62.5% of the total respiration customarily attributed to the phytoplankton. Failure to correct for bacterial respiration results in "net photosynthesis" values that are erroneously low by the same amount.

Large changes commonly occur in the bottled populations during a two-day light and dark bottle experiment: in 22 routine experiments diatoms in the light bottle increased an average of 264%/day and the total population increased 72%/day, while in the dark bottle flagellates and the total population usually decreased slightly. Respiration and net and gross photosynthesis per unit volume of water are therefore probably not the same inside and outside the bottles. The rapid multiplication of diatoms in the light bottle is due to an accelerated regeneration of nutrients by the bacteria attached to the bottle walls, as shown by comparison of diatom growth in water previously conditioned by bacteria with growth in water not so conditioned.

The light and dark bottle or dissolved oxygen method of Gaarder and Gran (1927) has long been a standard procedure for estimating the rate of plant production in the sea. Since Ryther (1954) has shown that the alternative method of Steemann Nielsen (1952), based on the uptake of radioactive carbon, actually measures net photosynthesis but not gross photosynthesis, the older method re-emerges as the only field technique for the direct measurement of gross production. This method has the added appeal of simplicity of equipment and procedure.

While other means of estimating production, *e.g.* that of Ryther and Yentsch (1957), show promise, the light and dark bottle method deserves reappraisal as the method on which most of our existing estimates of phytoplankton production have been based and because of its continued use. As employed in the study of wild populations in a natural state, the

method provides measurements of respiration and of net and gross photosynthesis that are useful only if they represent the rates of these processes outside the bottles. How they fail to do so in two respects is the subject of this report.

I. THE MISINTERPRETATION OF RATES OF RESPIRATION AND NET PHOTOSYNTHESIS

In the routine procedure, dissolved oxygen is measured at the start of the experiment, and bottles, generally of 200–400 ml capacity, are filled and submerged in the body of water being studied. Light is admitted to one bottle and excluded from the other. After a period of one or two days or longer, the dissolved oxygen content of the bottled waters is determined. Oxygen increase in the light bottle is interpreted as a measure of carbon assimilated in excess of respiration of the bottled community ("net photosynthesis"), oxygen decrease in the dark bottle as respiration, and the algebraic sum of these changes (or the difference in oxygen concentration in the two bottles at the end of the experiment) as equivalent to the total assimilation of carbon (gross photosynthesis). The application of the results to the population of the surrounding

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water mass assumes 1) that the total respiration in the bottle is the same as in an equal volume of water outside, and 2) that phytoplankton respiration in the bottle is to total bottle respiration as phytoplankton respiration outside is to total respiration outside. But if, in fact, an important part of the respiration measured is a bottle effect—the result of an agency not operative outside—then the value obtained will be greater than the respiration of the natural plankton assemblage of the open water, and the ratio of phytoplankton respiration to total respiration will not be the same in the bottle as it is in the surrounding water. Moreover, if this additional respiration occurs in the light bottle as well as in the dark, the estimate of net photosynthesis (as measured above) will be erroneously low by the same amount. The importance of measuring net photosynthesis is that this represents the increment of material synthesized in excess of the metabolic needs of the population and hence the amount that can be sacrificed (through sinking, grazing, and other losses) without change in the standing crop.

Bacterial growth has long been known to be a function of surface area (*e.g.*, ZoBell and Anderson 1936). Allusion to its possible importance in the light and dark bottle method is occasionally made (*e.g.*, Ryther 1956), and calculations show that the interior of the type of bottle used in the method presents a far greater surface area than the collective surface area of the contained phytoplankton population. (Using an ordinary 250 or 300 ml reagent bottle and assuming a population of spherical cells of 10 μ diameter at a concentration of one million per liter, the glass surface area is about 300 times that of the cells.) A large bacterial growth on the bottle walls can be expected, and the respiration of this population might therefore produce "bottle effects" as hypothesized above and erroneous values for phytoplankton respiration and net photosynthesis. An experiment was designed to determine what fraction of the respiration as measured in the bottles is bacterial.

A sample of sea water (salinity 28–32‰) from the end of the Laboratory pier in lower Narragansett Bay was divided in two parts. One portion was put directly into light and dark bottles. The other was passed through a Millipore Filter to remove all phytoplankton, bacteria, and other particles larger than 0.5 μ , inoculated from a bacterial culture of natural sea water in nutrient broth, and then put into light and dark bottles. Because of the number of measurements to be made, two bottles of each type were prepared. The resulting array, consisting of light and dark bottles of sea water with its natural phytoplankton population and associated bacteria and of light and dark bottles of sea water containing bacteria but no phytoplankton, was then submerged at a depth of two feet at the end of the pier for a period of two days. Initial and final determinations were made of dissolved oxygen (Winkler), bacteria (plate counts), and phytoplankton numbers (by counts of unpreserved, unconcentrated aliquots in a Sedgwick-Rafter cell). Before removing samples for bacterial counts from the bottles, the interior glass surfaces were scrubbed with a rubber policeman to dislodge attached bacteria, and three replicates of each of four appropriate dilutions of each sample were then incubated for seven days at room temperature. Oxygen uptake per bacterial cell per hour (m) in the dark filtered water was computed by the formula of Buchanan and Fulmer (1930, p. 154),

$$m = \frac{2.303 S \log b/B}{t(b - B)},$$

where S is the total amount of oxygen consumed (ml/ml) in time t (hours), B the number of bacteria per ml at the beginning of the experiment, and b the number after time t . This bacterial respiratory rate was then applied to the concentration of bacteria in the dark bottle of natural sea water, using the same equation and solving for S to give the fraction of the total respiration that was due to bacteria and by difference the true phytoplankton respiration.

Three points in the technique require brief mention before proceeding to the results. (1) Initial difficulties with film-forming or "spreader" organisms that grew rapidly and inhibited the development of other surface colonies were overcome by using ZoBell's Medium 2216

TABLE 1. *Changes in dissolved oxygen, bacteria, and phytoplankton in light and dark bottles of natural sea water and of Millipore filtered sea water inoculated with bacteria; respiration rates of bacteria and phytoplankton*

(Diatoms + flagellates = total population.)

	O ₂ ml/ ml $\times$ 10 ⁻³	Bacteria per ml	Dia- toms per ml	Flag- el- lates per ml
<i>Expt. 1. Forty-six hours, submerged off Laboratory pier, 14.8°C (Oct. 23-25, 1957)</i>				
<i>Filtered water</i>				
Initial	5.34	75 $\times$ 10	0	0
Final, light	5.17	79 $\times$ 10 ⁴	0	0
Final, dark	5.19	71 $\times$ 10 ⁴	0	0
Bacterial respiration, ml O ₂ /bacterium/hr.: light = 32.5 $\times$ 10 ⁻¹² , dark = 31.2 $\times$ 10 ⁻¹²				
<i>Natural water</i>				
Initial	5.32	91 $\times$ 10 ²	15	510
Final, light	5.37	26 $\times$ 10 ³	128	302
Final, dark	5.28	20 $\times$ 10 ³	57	213
Total respiration	0.04			
Bacterial respiration	0.02			
Phytoplankton respiration	0.02			
% respiration due to bacteria = 50.0%				
<i>Expt. 2. Forty-eight hours, submerged off Laboratory pier, 11.4°C (Nov. 13-15, 1957)</i>				
<i>Filtered water</i>				
Initial	5.85	13 $\times$ 10 ²	0	0
Final, light	5.55	47 $\times$ 10 ⁴	0	0
Final, dark	5.50	52 $\times$ 10 ⁴	0	0
Bacterial respiration, ml O ₂ /bacterium/hr.: light = 79.3 $\times$ 10 ⁻¹² , dark = 85.2 $\times$ 10 ⁻¹²				
<i>Natural water</i>				
Initial	5.75	64 $\times$ 10 ²	28	224
Final, light	5.86	34 $\times$ 10 ³	24	230
Final, dark	5.67	24 $\times$ 10 ³	11	102
Total respiration	0.08			
Bacterial respiration	0.05			
Phytoplankton respiration	0.03			
% respiration due to bacteria = 62.5%				

Expt. 3. Forty-six hours, in Laboratory, 21.0°C (Feb. 26-28, 1958)

<i>Filtered water</i>				
Initial	5.52	23 $\times$ 10 ²	0	0
Final, light	4.60	17 $\times$ 10 ⁴	0	0
Final, dark	4.84	17 $\times$ 10 ⁴	0	0
Bacterial respiration, ml O ₂ /bacterium/hr.: light = 78.0 $\times$ 10 ⁻¹² , dark = 56.2 $\times$ 10 ⁻¹²				
<i>Natural water</i>				
Initial	7.34	20 $\times$ 10 ³	3875	632
Final, light	6.46	50 $\times$ 10 ⁴	5689	255
Final, dark	6.14	73 $\times$ 10 ⁴	3314	136
Total respiration	1.20			
Bacterial respiration	0.51			
Phytoplankton respiration	0.69			
% respiration due to bacteria = 42.5%				

(ZoBell 1941) containing:

"Aged" sea water	1000.0 ml
Bacto-peptone	5.0 g
Ferric phosphate	0.1 g
Bacto-agar	15.0 g

(2) Inoculations were made by single loopful from a nutrient broth. This method should yield a more representative bacterial population than might be obtained by mechanically picking prominent surface colonies. The ratio of sea water to broth and the incubation time were adjusted empirically to yield an inoculum that would give an initial bacterial population in the filtered water of about the same concentration as the natural population in the untreated sea water.

(3) Millipore filtered water and autoclaved water, inoculated, showed the same rate of bacterial respiration in a two-day experiment, in spite of the fact that all particulate matter larger than 0.5 μ had been removed from the filtered sample.

The experiment designed to distinguish bacterial from phytoplankton respiration as outlined above was done at the following mean temperatures: 21.0°, 14.8°, 11.4°, 6.8°, 3.9°, 3.3°, and 1.0°C. The results of the experiments at the three highest temperatures are given in Table 1. In the remaining experiments (below 11°C) low bacterial numbers and rates of respiration combined to yield results that were either theoretically impossible or within the error of measurement.

TABLE 2. Comparison of rates of phytoplankton respiration and net photosynthesis ($\text{ml O}_2/\text{L/day}$) in light and dark bottle experiments when (A) respiration as measured includes bacterial respiration, and when (B) bacterial respiration is deducted from total respiration

Experiment	Respiration		Net photosynthesis	
	A	B	A	B
1	.02	.01	.025	.035
2	.04	.015	.055	.08
3	.60	.395	-.44	-.185

If there are bacterial effects of any appreciable magnitude in the bottles, it is important to know whether they are the same in the light and the dark. Vaccaro and Ryther (1954) have shown that in light and dark bottle experiments as commonly conducted, the effects of sunlight on bacterial growth and respiration are negligible. This conclusion was corroborated in a preliminary experiment (46 hr, submerged off the pier at 16°C) in which rates of bacterial growth and respiration in Millipore filtered inoculated water were nearly identical in light and dark bottles. It is further supported in the experiments reported in Table 1: in the filtered water bacterial growth and respiration were substantially the same in the light as in the dark, except in one instance (Experiment 3) in which respiration was greater in the light.

Experiments 1 and 2 were conducted off the Laboratory pier at the prevailing water temperatures and approximately at the time of the annual phytoplankton minimum. In both cases the population consisted principally of unidentified flagellates of less than $15\ \mu$ with small numbers of several diatom species, especially *Skeletonema costatum*, *Corethron hystrix*, *Thalassionema nitzschioides*, *Asterionella japonica*, and *Chaetoceros* sp. Experiment 3 was carried out during the annual winter diatom flowering with a population dominated by *Detonula cystifera* and *Skeletonema costatum*. Because of the inhibition of respiration at low temperatures observed in other experiments, this experiment was

conducted in the laboratory at room temperature, in the light of a north window.

While the total respiration in Experiment 1 was admittedly just equal to the sum of the accepted errors of oxygen measurement in the initial sample and the dark bottle (each $\pm 0.02\ \text{ml/L}$) and hence is of questionable significance, in all three experiments the calculated respiration of the bacteria in the natural water proved to be a considerable fraction of the total respiration (42.5–62.5 %).

Failure to distinguish bacterial respiration from that of the phytoplankton leads to misinterpretations of light and dark bottle data. The experiments described above provide examples of such misinterpretations, as shown in Table 2. While gross photosynthesis is not affected, the correction for bacterial respiration necessitates an equivalent addition to the value for net photosynthesis.

Our experiments indicate that the importance and feasibility of such a correction increase with temperature. With the methods here employed, below 10°C a correction is apparently not possible and any resulting error is probably negligible, while at about 20° bacterial respiration is readily measurable, and failure to correct for it results in an appreciable overestimation of phytoplankton respiration and underestimation of net photosynthesis.

II. POPULATION CHANGES WITHIN THE BOTTLES

If the contained populations in a light and dark bottle experiment undergo significant changes in numbers that do not mirror the course of events in the open water, the metabolic rates measured in the bottles cannot be considered representative of changes occurring outside.

Phytoplankton counts were made at the beginning and at the end of the two-days' exposure in 22 light and dark bottle experiments. These were done in 400-ml citrate bottles at a depth of two feet, during the months June–November inclusive in 1954 and 1955, at mean temperatures ranging from 9.0° to 24.3°C . In 16 instances counts of the open water populations

were made one week after the beginning of the experiment, as part of a routine sampling program. A comparison of population changes (increases or decreases) in the bottles (two days) with those in the open water (seven days) shows that in both bottles the changes in numbers of diatoms, in numbers of flagellates, and in total cells were usually in the opposite direction from the changes in the open water. In the light bottle, for each of these categories the changes in the opposite direction were two to three times as many as the changes in the same direction. In short, the direction of change (increase or decrease) in the bottles paralleled changes in the open water in only a small minority of the experiments.

The percentage rates of change per day in the 22 experiments have been averaged. In the light bottle, diatoms showed a mean increase of 264%, flagellates increased by 4.2%, and the total population 72% per day. In the dark bottle, mean daily changes were: diatoms, +8.7%; flagellates, -14%; total cells -14%. In the light bottle, practically all of the diatom species represented took part in the increase, including *Skeletonema costatum*, *Chaetoceros* *terres*, *C. curvisetus*, *Leptocylinndrus minimus*, *Nitzschia closterium*, *N. reversa*, *Thalassiosira rotula*, *T. nana*, *T. decipiens*, *Rhizosolenia fragilissima*, and *Asterionella japonica*. In every experiment but one, diatoms increased in the light bottle; the changes in the numbers of diatoms in the dark and of flagellates in the light or the dark were not so consistent in the direction of change. Similar observations made by Smayda (1957) gave an average diatom increase in the light bottle of only 53%, but the two sets of data lead to the same general conclusion: there is usually a significant increase in the diatoms and the total population in the light bottle, while in the dark bottle the flagellates and the total population usually decrease slightly. At the end of a two-day experiment, the phytoplankton concentration in the light bottle is apt to be considerably greater than that in the dark bottle or in the water originally sampled. When this is true, total respiration in the light bottle is prob-

TABLE 3. *Phytoplankton concentrations in light and dark bottle experiments and percentage changes per day*

Date 1955	Cells/ml.			% change/day		
	Dia- toms	Flag- el- lates	Total	Dia- toms	Flag- el- lates	Total
16-18 Initial	14,963	969	15,932			
Aug. Final, light	27,370	1,196	28,566	+41	+11	+40
Final, light + glass wool	35,882	1,502	37,384	+70	+28	+64
Final, dark	36,648	1,388	38,036	+72	+22	+69
23-25 Initial	343	916	1,259			
Aug. Final, light	762	1,057	1,819	+61	+7.8	+22
Final, light + glass wool	911	976	1,887	+83	+3.3	+24
Final, dark	133	473	606	-31	-24	-26
29-30 Initial	24,637	765	25,402			
Aug. Final, light	40,619	1,295	41,914	+65	+69	+65
Final, light + glass wool	61,183	976	62,159	+115	+28	+114
Final, dark	15,739	1,394	17,133	-36	+82	-32
14-16 Initial	71	991	1,062			
Sept. Final, light	227	1,269	1,496	+108	+14	+20
Final, light + glass wool	826	1,037	1,863	+523	+2.8	+38
Final, dark	38	935	973	-23	-2.8	-4.2

ably not the same as in the dark bottle, and both net and gross photosynthesis in the light bottle are greater than in an equal volume of the water sampled at the start of the experiment. Thus the population changes within the bottles produce errors in all three of the rate measurements obtained by the light and dark bottle method. To attempt quantitative estimates of these effects would involve assumptions that do not appear warranted, such as equating the metabolic rates per cell of species differing widely in size and taxonomic position.

The diatom increase in the light bottle appears to be a function of the glass surface area of the bottle interior. This was shown in four light and dark bottle experiments in which a second light bottle was added, containing a wisp of glass wool calculated to increase the glass surface area approximately three-fold without significantly decreasing the bottle capacity. The results are shown in Table 3. The added glass wool consistently enhanced the diatom increase, and thereby that of the total population, in spite of inconsistent

TABLE 4. *Phytoplankton concentrations in light bottle experiments in sea water previously conditioned by bacterial growth as compared with sea water not so conditioned*

(A) Bacterial conditioning 75 hours; duration of experiment after phytoplankton seeding 47 hours (17-19 March, 1958), submerged off Laboratory pier, 3.2°C.

(B) Bacterial conditioning 46 hours; duration of experiment after phytoplankton seeding 46 hours (2-4 April, 1958), in laboratory, 23.6°C.

	Cells/ml			% change/day		
	Dia-toms	Flag-el-lates	Total	Dia-toms	Flag-el-lates	Tot-al
(A) Initial	990	115	1,105			
Final, not conditioned	1,376	130	1,506	+19	+6.5	+18
Final, conditioned	2,066	113	2,179	+54	-0.9	+49
(B) Initial	801	264	1,065			
Final, not conditioned	6,943	59	7,002	+383	-39	+279
Final, conditioned	11,122	92	11,214	+644	-33	+476

effects on the changes in flagellate numbers. (We are unable to account for the large increase in diatoms in the dark bottle of the 16-18 August series.)

The correlation of diatom growth with glass surface area may be related to the leaching of nutrient substances, particularly silicate, from the glass. However, the rapid multiplication of bacteria associated with the bottle surface suggested that the diatom growth might be due to the release of additional nutrients from particulate matter and organic complexes in solution, brought about by the heightened bacterial activity. Such an accelerated regeneration would allow the bottle phytoplankton to tap nutrient stores not immediately available to the open water population.

This hypothesis was tested by comparing phytoplankton growth in water previously conditioned by bacteria with growth in water not so conditioned. A sample of freshly collected sea water was Millipore filtered to remove phytoplankton and bacteria, and dispensed into four 280-ml experimental bottles. Two bottles were inoculated with a loopful from a broth culture of marine bacteria, and all four bottles were set in the dark for three days at room temperature. All bottles were then refiltered and seeded equally from a

dense suspension of phytoplankton (with its natural complement of associated bacteria) concentrated by Millipore filtering from a fresh sample of sea water. The resulting phytoplankton concentration was determined by similarly seeding a 280 ml aliquot of filtered water and counting immediately. The four bottles, all light, were then suspended off the pier for two days. Mean water temperature was 3.2°C, and the population consisted primarily of the diatom *Detonula cystifera*, with smaller numbers of *Skeletonema costatum*, microflagellates, *Thalassiosira nordenskioldii*, and other species. The results are shown in Table 4(A).

This experiment was repeated with two modifications. (1) Autoclaving was substituted for filtering at the beginning of the procedure and after the period of bacterial conditioning, in order to reduce the possibility of bacterial contamination. (2) Observations had shown that when *Skeletonema costatum*, numerically the most important phytoplankton species in Narragansett Bay, is transferred from the natural environment at 2-3°C to bottles at room temperature, it continues to multiply rapidly. This species dominated the plankton when the second experiment was conducted. The experiment was done in the laboratory at a temperature that would simulate the summer maximum and accelerate the metabolism and growth of the phytoplankton and bacteria. Aside from *Skeletonema*, the principal constituents of the seeding population were microflagellates, *Thalassiosira nordenskioldii*, *Chaetoceros* sp., *Detonula cystifera*, and *Schröderella delicatula*. Phytoplankton counts two days later yielded the results presented in Table 4(B). Most of the increase in numbers was due to multiplication of *Skeletonema*.

In both experiments diatoms and the total population increased in the unconditioned water but increased considerably more in the water in which bacteria had been growing. These results are interpreted thus: the multiplication of diatoms in the unconditioned water is due, at least in part, to the regeneration of nutrients by bacteria growing attached to the bottle

wall after their introduction with the phytoplankton; in the conditioned water, this stimulus to diatom increase is augmented by an initial accumulation of nutrients released during the previous period of bacterial conditioning. The increase of diatom numbers and thereby total cells commonly occurring in the light bottle in a routine bottle experiment is attributed to an accelerated supply of nutrients brought about by an artificially increased bacterial activity.

If this interpretation is correct, it provides an artificially intensified illustration of a bacteria-phytoplankton relationship that exists under natural conditions in the open water. The development of a phytoplankton bloom increases the surface area available to the bacteria. The activity of the bacteria in turn increases the rate of supply of nutrients to the photosynthetic plankton, which is thereby stimulated to multiply further, thus providing additional bacterial substrate—an autoaccelerating process.

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Chlorophyll and Productivity in a Mountain River¹

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ABSTRACT

Chlorophyll was sampled on the shallow, rocky, canyon section of Logan River—a swift, clear, calcareous mountain river in the Middle Rocky Mountains of northern Utah. Chlorophyll extractions were made by immersing entire rocks supporting algae in acetone. The average quantity of chlorophyll per m² of bottom of the canyon section of Logan River was 0.30 grams. Downstream supplementary stations supported 3 to 4 times as much chlorophyll as the canyon section. No significant difference existed between means of chlorophyll samples in fall and late winter and between the upstream and downstream portions of the canyon section.

Annual gross primary production equal to about 1.2 kg per m² was estimated from the relation of chlorophyll to photosynthesis in light and dark jar experiments. A standing crop of 25 grams of dry plant biomass per m² was determined from the chlorophyll dry weight ratio of samples of the community. Average standing crops (dry wt.) of insects and fish were 5 and 1.6 per cent, respectively, of the producer biomass or average standing crop of algae.

Production of chlorophyll on artificial substrata (concrete rocks) showed no close relationship with either insolation or water movement within the range of values encountered in the Logan River.

INTRODUCTION

Investigation of primary production in streams and rivers has lagged behind similar investigations in marine and lacustrine environments. Recently, however, Odum (1956) has demonstrated methods that allow the estimation of productivity of most moving waters. Odum's method, unfortunately, is not satisfactory for determination of productivity in shallow, very rapid rivers as typified by Logan River. In this kind of environment the diurnal pulse in dissolved oxygen is caused primarily by changes in the saturation value related to diurnal temperature changes rather than by accumulation and depletion of photosynthetic oxygen. The investigation herein reported was primarily an exploration of a method possibly applicable to measurement

of primary productivity under the foregoing conditions. The relationship of photosynthesis to chlorophyll content in planktonic algae (Edmondson 1955, Ryther 1956b) suggested this exploration.

In addition to the measurement of productivity, the relation of chlorophyll to standing crop of algae and the effects of diffusion and insolation on chlorophyll distribution were investigated.

The importance of current photosynthesis in rapid rivers is, perhaps, greater than it is in other aquatic habitats. Attrition due to water currents is a constant process which removes primary food material and carries it away from the site of production. Brief measurements of pseudo-plankton or stream drift suggest that a large percentage of the net product of photosynthesis is lost downstream. Nowhere on the bed of Logan River is there evidence of important conservation of organic matter in the form of muck or plant detritus. Although quantities of leaves fall into the river, terrestrial

¹ Based on a thesis presented by the first author to the Department of Wildlife Management of Utah State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

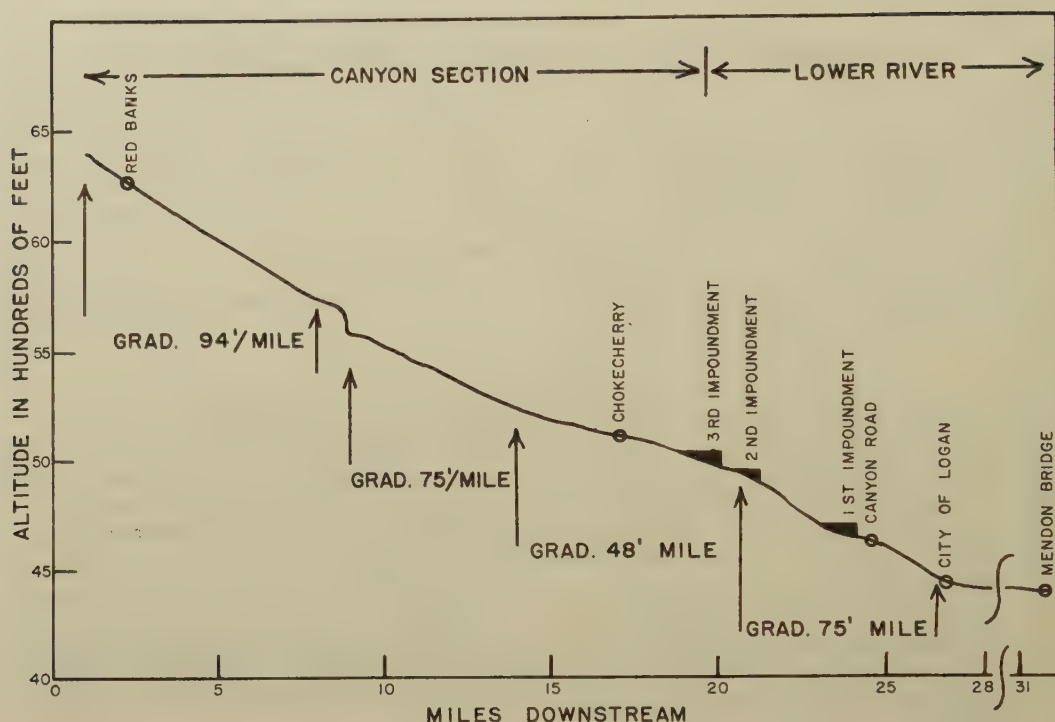


FIG. 1. Profile of Logan River showing gradients, stations, and sections referred to in the text.

contributions are actually unimportant. Little of the imported plant material reaching the canyon section of Logan River is arrested in downstream transport until it reaches the 3rd impoundment (Fig. 1).

The food habits of the dominant fishes of Logan River indicate a complete dependence on aquatic insect larvae (Fleener 1951, Zarbock 1951, Sigler 1951). Although the food of many North American stream insects has not been intensively investigated, work on the food habits of closely allied insects from British streams shows that algae are an important food of the herbivorous types (Badcock 1949, Jones 1949, 1950).

Provision of the basic grant (E-1435C) by the National Institutes of Health of the United States Public Health Service made the research possible. William J. Clark cooperated closely in the collection of most of the physical and chemical data. Dr. Francis Drouet identified reference collections of algae which were the basis for identifications in routine algal collections. We also thank the people at Utah State

University and elsewhere who provided valuable suggestions and criticisms.

MATERIALS AND METHODS

Chlorophyll was extracted by soaking rocks supporting algae in acetone. Bleaching of the chlorophyll solution during extraction was minimized by the use of closely covered containers and extraction at low temperatures. The magnitude of errors introduced by bleaching, incomplete extraction, turbidity of extracts and varying water content of extracts was assessed by supplementary experiments. Appropriate corrections were made (McConnell 1958).

Optical density of acetone chlorophyll extracts was measured at $664\text{ m}\mu$ with a Bausch and Lomb "Spectronic 20." The chlorophyll *a* equivalent to these optical densities was determined by reference to the relationship between optical density at $664\text{ m}\mu$ and the 3 wavelength determination prescribed by Richards with Thompson (1952). This relation proved to be very constant (Fig. 2).

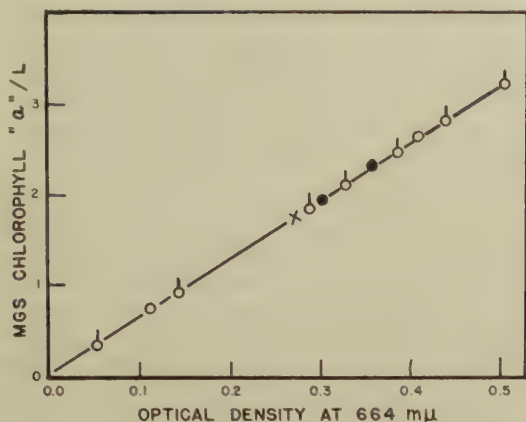


FIG. 2. Relationship between optical density and chlorophyll *a*. Light path is 22 mm. Algae extracted are: tailed circles, encrusting bluegreens with *Palmella*; open circles, *Palmella*; cross, *Vaucheria*; filled circles, *Oscillatoria*.

Comparisons made at a later date and using acetone extracts of marine algae indicate that chlorophyll determinations made at relatively wide band passes in this investigation were about 15 per cent lower than comparable narrow band measurements made as prescribed by Richards with Thompson (1952) because of the "running average effect" of the wide band measurements made on the "Spectronic 20."

Although varying in apparent color from the brown extracts of bluegreen algae to the bright green extracts of *Cladophora*, the absorption spectra of the acetone extracts of all communities encountered were similar (Fig. 3).

SAMPLING PROCEDURE AND DEVICES

The 18-mile length of the canyon section was divided into nine 2-mile units. Order of sampling and location of a transect line within each unit was determined by lot. Bottom materials touched by a rod dropped every 15 cm along the transect line were classified by size. Rocks for chlorophyll measurement (8 to 10) were also collected along each transect line at randomised points. The sampling was stratified by rock size in order that differences in amounts of chlorophyll per unit of bottom area occupied by rocks in 2 size categories could be detected. Stream bed area occupied by

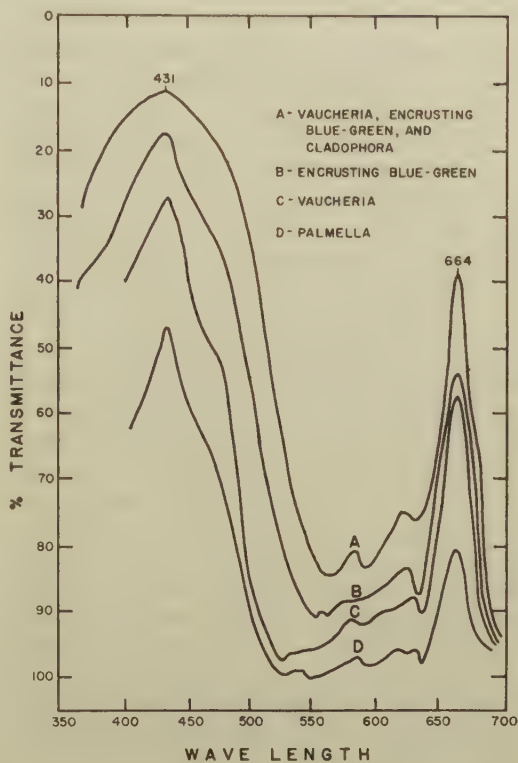


FIG. 3. Absorption spectra of acetone extracts of algae or groups of algae dominating in Logan River.

rock categories was considered to be the sum of the areas of vertical projections. A 25-cm upper size limit on rocks was dictated by the dimensions of the extraction container; however, supplementary experiments did not indicate a relation between chlorophyll per unit area and rock size when minimum dimensions were greater than 12 cm. The weighting of variances and means of sampling strata, and the estimation of the variance of the product: (Stream bed area occupied by chlorophyll bearing rocks) $\times$ (amount of chlorophyll per unit area) followed methods outlined by Snedecor's section 17.7 (1946).

Artificial substrata

To remove the effect of substratum type and shape on experiments concerning the relation between chlorophyll distribution and insolation, relative rate of diffusion, and downstream habitat changes, artificial

concrete "rocks" were used. Each "rock" was actually a cylinder of concrete 6 inches high and 9 inches in diameter with a wire loop embedded at the top.

Extraction of the chlorophyll contained in algae growing on the concrete "rocks" was accomplished by placing each in a closely fitting enameled pail with enough acetone to just cover the "rock."

Relative diffusion methods

The most tenable explanation for enhanced plant growth in moving waters is the increase in rate of renewal or removal of gases and dissolved solids in the water bathing the plants (Ruttner 1926, as quoted by Ruttner 1953. For this reason it is necessary to measure the total water movement occurring close to algae when assessing such diffusion effects. Total water movement determinations infer a measurement of turbulent micro-currents and alternating surges as well as orderly streamlined flow. All mechanical velocity or current meters in use today are incapable of measuring the micro-currents in intimate contact with algae. The majority are too bulky to be placed in the environment with which we are concerned, and in addition they only integrate micro-currents of a relatively large cross section of stream flow. Pitot tubes, besides being difficult to read in rapid, turbulent water, do not record alternating surges or turbulence as water movement. The ideal device to determine the rate of exchange of water molecules over a submerged surface would be one dependent on this very phenomenon. As an approach to this ideal, the rate of dissolution of sodium chloride tablets was used as an index of relative diffusion in this investigation.

The relative rate of diffusion over the surface of cylindrical concrete "rocks" was measured as the total loss by dissolution, of six, 2.25-gram sodium chloride tablets during a 1-minute immersion when placed at 6 standard positions over the surface of the "rock." The entire salt tablet was held within 8 mm of the substratum by adjustable spring wire tongs. The tablet was streamlined in relation to the main current.

The original tablet weights as manu-

factured are standardized within close tolerances so that any difference in weight may be considered due to dissolution. Series of tablets placed in individual, identical containers of still water sustained almost identical weight losses during an immersion of several minutes.

To investigate further the precision of the method, 2 series of replicated tests were conducted under the conditions of actual use. In a lined canal having smooth, non-turbulent flow, mean weight of salt loss for 6 replications of 4 tablets each had a coefficient of variation of 3 per cent. The velocity here was 0.6 meter per second. At another situation on Logan River itself, immediately downstream of a cascade where surface velocities exceeded a meter per second and the flow was very turbulent, 11 replications of 4 tablets each yielded a coefficient of variation of 6 per cent. The 4 tablets in the above tests were placed at the 4 side positions (0, 90, 180, and 270 degrees) of the cylindrical concrete "rock" used for the measurements.

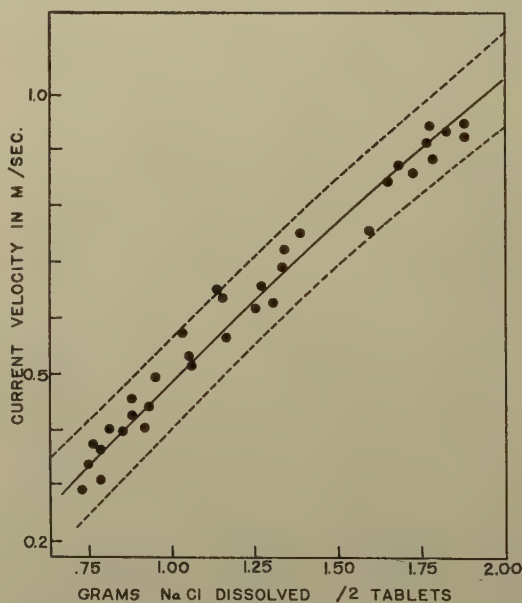


FIG. 4. Relationship between grams of NaCl dissolved and velocity of water in which two 2.25-gram tablets were immersed for 1 minute. The least squares regression line is bracketed by lines enclosing the prediction interval at 95 per cent confidence.

As would be expected, salt loss by dissolution bears an erratic relationship to velocity meter readings in turbulent parts of the river. In smooth flow, as in a lined canal, the relationship is close enough to allow the use of salt dissolution as a measure of main current velocity. At velocities from 0.3 to 0.9 meters per second, prediction intervals at 95 per cent confidence (Ostle 1954) were all within 0.08 meters per second (Fig. 4). Corresponding values of salt loss for 2 tablets and velocity of water in which they were immersed were obtained by mounting the tablets ahead of an Atlas current meter in such a fashion that the normal flow through the meter was not impeded. Because each of several improvements in the calibration technique gave a worthwhile improvement of precision, it is felt that much of the present variability in the velocity-salt loss relationship could be removed by additional improvement in calibration technique.

Vertical gangs of salt tablets mounted on staffs at 2-inch intervals proved useful in exploring velocity distribution with depth.

Duration of insolation methods

With the exception of changes due to solar altitude and cloud conditions, the chief cause of differences in amount of light reaching plants on the bottom of Logan River is shade formed by mountains and trees. Sunlight intensities measured on the river bottom varied little from place to place when surface intensities were equal.

The measurement of duration of insolation, the complement of shade, was accomplished by photographing 140 degrees of the celestial hemisphere at each point where a measurement was desired. From one vertical photograph, objects that would intercept insolation at some time of the year could readily be seen when an overlay of sun courses was oriented on the photograph. The overlay consisted of courses the sun would follow on dates coincident with 5°-intervals of noontime elevation of the sun as observed from a latitude of 42° (Fig. 5). Data for the construction of the overlay were obtained from a sun azimuth chart published by the United States Hydrographic Office, Washington, D. C., and from Rusts

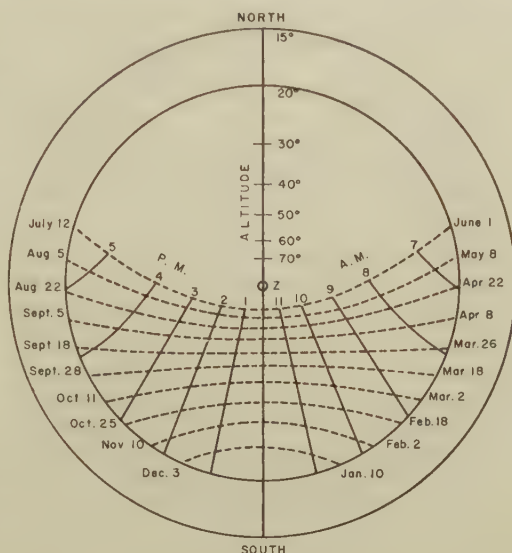


FIG. 5. Details of transparent overlay placed over photograph of sky and shade forming objects to determine the duration of insolation. Dashed lines are sun courses at 5 intervals of noontime elevation (local civil time). Angular altitudes on the celestial hemisphere are given on the N-S line. Dates around lower half of the 20° circle are those on which the sun follows the course adjacent indicated by a dashed line. Each sun course is followed 2 times a year.

Line of Position Chart (Weems 1941). Duration of insolation between any 2 dates on the overlay was computed by counting the hours along the 2 courses when the background for the course line consisted of open sky on the photograph. Figure 6 is an interpreted tracing of 2 typical photographs showing the open and occluded areas of the sky that are the basis for calculation. The open sky periods for both dates were then averaged and multiplied by the number of days represented by the interval between sun courses. Total insolation for any desired period consisted of the sum of hours of insolation in each of the 5° intervals in the period.

The actual photograph was taken as a negative on 5 x 7 contact printing paper. A simple, handmade, wide angle, pinhole camera, built about a 5 x 7 cut film holder, was used to take the photographs. A focal length of about 20 mm gave at least 140° coverage of the celestial hemisphere. A minus 4 diopter lens was placed over the

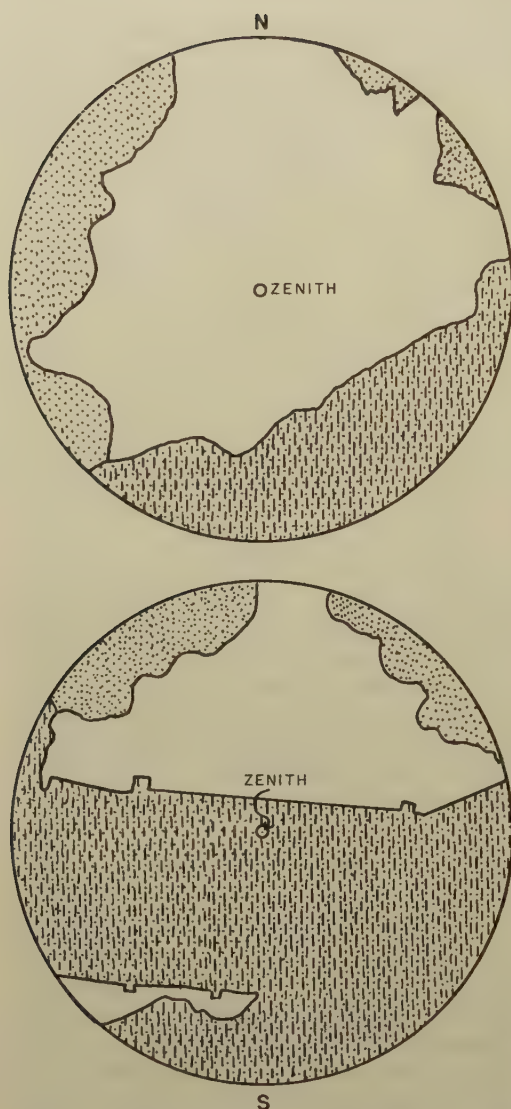


FIG. 6. Interpreted tracing of 2 heavy shade situations as recorded on vertical, wide angle photographs. Dotted areas represent brush; hatched areas represent mountains and a bridge over the point for which a potential insolation measurement was desired.

0.5 mm pinhole to reduce distortion. When light rays forming a point image are parallel, as in a pinhole camera, negative lenses have a "focusing" effect on oblique rays of light reaching the pinhole which causes them to be focused closer to the center than they would normally be focused. The effect decreases as the rays approach the perpendicular.

Orientation points necessary to properly place the overlay on the photograph were the sky zenith and true north. The zenith was obtained as a photograph of a light bulb precisely over the pinhole taken while the camera and film plane were perfectly level as in actual use. The position of the zenith was then transferred to other photographs by lining up the border (actually a pencil tracing of the outlines of the film rabbets when the paper was in place) of the zenith standard with the photograph.

True north in mountainous country was easily located by taking a compass bearing on a distant, sharp mountain peak that would appear in the photograph. In flatter country true north was located by including the sun in the picture. By recording the local civil time at which the photograph was taken, an azimuth table could be entered. With knowledge of the sun's azimuth it was then a simple matter to compute true north.

Circles of equal celestial altitude were constructed on the overlay by photographing marks of known angular altitude on a wall while the pinhole camera was level and then measuring the equivalent distances from the zenith on the photograph.

Productivity methods

Efforts were made to apply Odum's (1956) methods of estimating productivity by the diurnal oxygen pulse of a stream. The data obtained from the canyon section were very erratic and not subject to interpretation. Oxygen values were never more than 0.4 mg/L away from saturation, while the range in duplicate samples often approached one-half of this figure.

In the deep, slowly flowing stream produced by the third impoundment on Logan River, diurnal changes exceeding 5 mg/L were measured, and duplicate samples agreed closely. A measurement of the productivity of that part of the river was made on a day in July. The yearly average was estimated by arbitrarily assuming the lowest winter rate to be about 10 per cent of the July rate.

To gain an idea of the order of magnitude of photosynthesis as related to chlorophyll in Logan River, several series of light and

dark bottle type experiments were conducted. The experiments were made with full realization of the probability that running water communities do not photosynthesize at a normal rate when sealed in containers of still water.

The winter series of light and dark bottle experiments were conducted in gallon jars fitted with paraffin exclusion plugs. Each plug was provided with a stoppered, half-inch hole through which the water could be siphoned. Rocks supporting typical growths of algae were placed in the jars and sealed. The dark jars were placed in a covered box while the light jars were placed on an improvised bench at the stream side. Temperatures were held close to those of the river by banking the shade side of the jars with snow. Light values were the same as those on the river bottom. The summer series of experiments utilized quart mason jars sealed under the water surface. Jars were tethered to a plank bridge over a canal and allowed to rest on the concrete bottom. The light, temperature, and water chemistry of the canal were identical with the Logan River itself.

Light experiments ran from 2 to 3 hours, dark experiments from 3 to 16 hours. The longer dark experiments led to respiration rates much lower than the short ones; therefore, results only from short experiments were used. The decreased respiration rate of long experiments was associated with the observed death or distress of hundreds of small insect larvae that had crawled from the algae. All respiration rates were chlorophyll based (oxygen consumed per mg of chlorophyll); for obvious reasons such rates can only be approximations. In addition to the light and dark jars, blanks of canal water only were exposed to the same handling and environmental conditions. Oxygen concentrations of these jars were the basis for determining differences in concentration in the others. No measurable changes in oxygen concentration occurred in the blanks.

Rates from each experiment are averages of data from 3 to 6 jars. No attempt was made to determine the productivity rate from jar to jar because of the difficulty of placing similar quantities of material in

each jar and of having the same proportion of chlorophyll in the shadow of the rock on which it was growing.

Chemical, temperature, and light methods

Determinations of dissolved solids were made by Utah State University Soils Laboratory. Appropriate standard methods were used. Oxygen values were determined by the unmodified Winkler method. Turbidities were measured with a Hellige turbidometer. Water velocities were determined with an Atlas current meter. Temperatures were taken with maximum-minimum thermometers. Relative intensities of light were measured with a Weston photographic exposure meter.

DESCRIPTION OF THE LOGAN RIVER COMMUNITY

Logan River is a clear, shallow, rapid stream arising in a portion of the Rocky

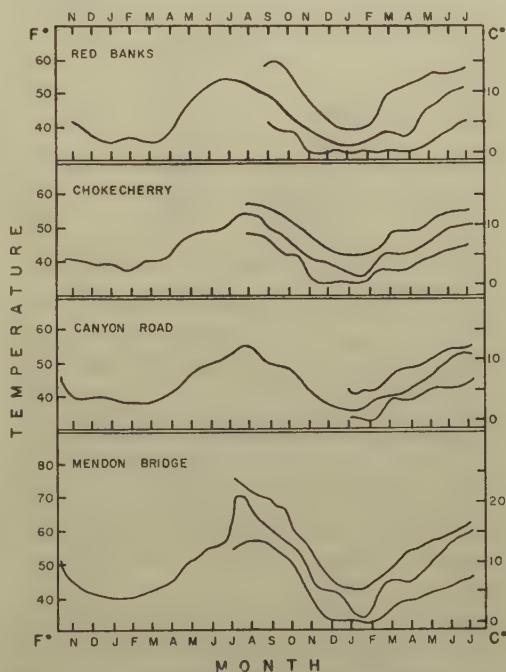


FIG. 7. Water temperatures at 4 stations on Logan River. Center line is smoothed curve of temperatures taken at random times during the day at intervals of 10 to 20 days. Lines above and below center line are smoothed curves of maximum and minimum temperatures. Measurements were started in 1955 and ended in 1957.

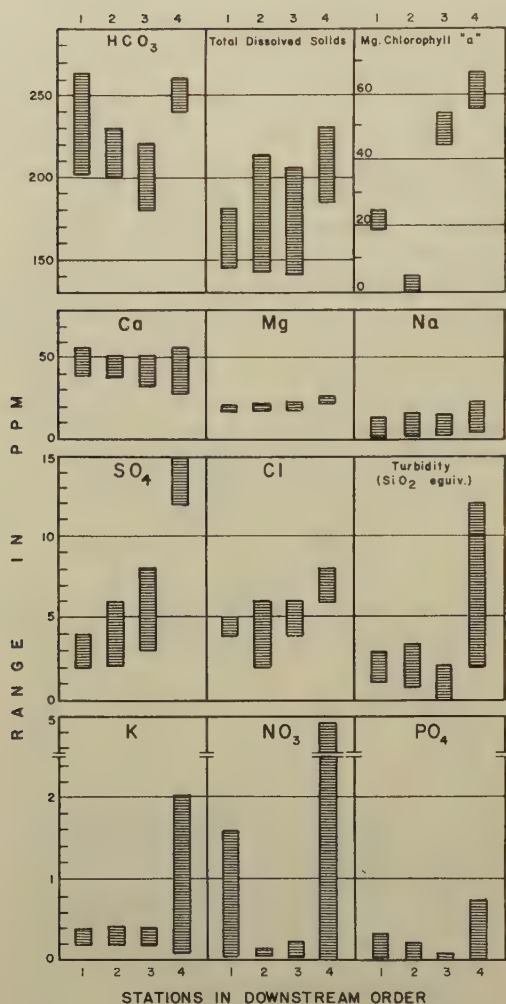


FIG. 8. Downstream changes in dissolved solids, turbidity, and chlorophyll. Ranges in dissolved solid concentration are based on 3 series of determinations made in September, January, and April 1956-1957. Turbidities are based on measurements made at ten-day intervals from August to March 1956-1957. Chlorophyll values are 95 per cent confidence belts for average amount extracted from concrete rocks immersed at stations indicated from August 1956 to March 1957.

Mountains in northern Utah and terminating in Bear River about 30 miles from Great Salt Lake. The canyon section, the portion of greatest interest in this paper, may be described as an almost continuous, uniform, flat riffle varying from 0.2 to 0.5 meters in depth and about 11 meters in width. Surface velocities from August to March range

from 0.6 to 1.2 meters per second. These velocities are doubled during the spring and early summer runoff. Though having a steep gradient (Fig. 1) the river bed is stable. Course rubble and small boulders comprise about 75 per cent of the bottom materials. The remainder is divided equally between large boulders and gravel.

Turbidities are usually lower than 5 ppm (SiO_2 equivalent) but briefly may rise to 40 ppm in high water. Averages and extremes of temperature are shown in Figure 7. Preponderant ions are summarized in Figure 8.

Fish are the only vertebrates of importance within the food web of the river. Species present are: mountain whitefish, *Coregonus williamsoni* Girard; brown trout, *Salmo trutta* Linnaeus; rainbow trout, *Salmo gairdneri* Gibbons; cutthroat trout, *Salmo clarki* Richardson; Utah sculpin, *Cottus bairdi semiscaber* Cope; and rarely the small fin redbreast shiner, *Richardsonius balteatus hydroflox* Cope (Fleener 195, Sigler 1951). The summer standing crop of unstocked fish species determined by electro-fishing was 21 kg per hectare (live weight) or 19 pounds per acre in 1954 (unpublished data, Utah State Fish and Game Department).

Insect larvae are the preponderant invertebrates living in Logan River. Data of Hales (1955) can be used to calculate the average density of the most important orders of insects. Based on square-foot bottom samples from 3 representative stations sampled at several times of the year, the combined wet weight of Diptera, Ephemeroptera, Trichoptera, and Plecoptera was about 6 grams per m^2 . The order of abundance of the 4 dominant orders of insects is open to question.

Other invertebrates do not approach the numbers and mass of insect larvae in Logan River. Free living nematodes and rotifers are common, but all others not discussed here are not present or only occasional.

The principle producers and chlorophyll bearers of the Logan River are sessile algae. Dominating genera of the canyon section were *Phormidium*, *Shizothrix*, *Palmella* (*Hydrurus*), *Nostoc*, and *Prasiola*. In the lower

Logan River *Vaucheria*, *Cladophora*, diatoms, and occasionally bryophytes were the most important plants.

RESULTS AND DISCUSSION

Amount and distribution of chlorophyll

The average amount of chlorophyll *a* present per m² of the bed of the canyon section of Logan River was 0.30 grams (0.25–0.35 at 95 per cent confidence). Most of this was contained in algae supported by rocks having a minimum dimension of 12 cm or greater. The 95 per cent confidence band for chlorophyll *a* per m² of river bed occupied by this size category of rocks extended from 0.30 to 0.40 grams. Those rocks having a minimum dimension between 2.5 and 12 cm supported between 0.15 and 0.25 grams per m² at the 95 per cent confidence level. Less than 6 per cent of all chlorophyll was supported by bottom material having a minimum dimension smaller than 2.5 cm. No trend in average chlorophyll concentration with distance from the river source within the canyon section was observable.

The net effect of short-term changes in algal abundance during unsampled periods on the estimate of the average standing crop of chlorophyll was not determined precisely. Evidence from several sources, however, gives no reason to suspect that such knowledge would cause an important change in the estimate. The amounts of chlorophyll on 5 concrete "rocks" (in mid-July) that had been on the river bed during the spring runoff were within the range of comparable values of chlorophyll from identical artificial substrata removed about the same time the late winter chlorophyll measurements of the natural substrata were made.

The fall series of chlorophyll transects was made over a period of 60 days, that of the late winter series was 30 days. No trend of increasing or decreasing quantity of chlorophyll per unit area was recorded during either period. The difference in mean amount of chlorophyll per m² of river bed occupied by large rocks (the dominant chlorophyll-bearing substrata) between that measured in the fall and that of the late

winter series of transects was not significant at confidence levels as low as 50 per cent.

Continuous observation of the algal community of the canyon section gave no indication of a large bias in the estimate of the chlorophyll *a* contained in the community. Peaks and low points in the standing crop, though occurring, were not of long duration and seemed to balance out. An upsurge of algae during the decline of the runoff was followed by a general reduction in standing crops in August and September. Brief chlorophyll measurement at 4 representative stations in August yielded a mean quantity of 0.14 grams per m² in the canyon section. A peak standing crop occurred in early winter. Although not measured, it did not appear possible that the contained chlorophyll could have exceeded the average estimate reported by 50 per cent.

Two stations on the lower Logan River supported a much higher standing crop of algae and consequently of chlorophyll than did the canyon section. This is probably due to reduction of the bed load by 3 impoundments in the portion of the lower river with a steep gradient and to the lack of movement of potential molar material after the river reaches the valley. Two measurements, fall and spring, a short distance below the first impoundment (Canyon Road) yielded values of 0.75 and 1.42 grams per m², respectively. The higher spring value here reflects heavy winter algal growth in lower portions of the river in which anchor ice does not develop. A canal diverted from the first impoundment supported 1.03 grams of chlorophyll per m² during mid-winter. The benthic algae at Mendon Bridge contained 0.62 grams of chlorophyll per m² in one early spring measurement, the time of apparent maximum standing crop there. The preceding values may be compared to benthic determinations of 0.49 grams per m² on a coral reef (Odum and Odum 1955) and 2.95 grams at Silver Springs, Florida (Odum 1957a).

The range of values of total plankton chlorophyll per m² of surface in 5 Wisconsin lakes was 15 to 65 mg (lakes areas: Kosminski 1938; chlorophyll content: Man-

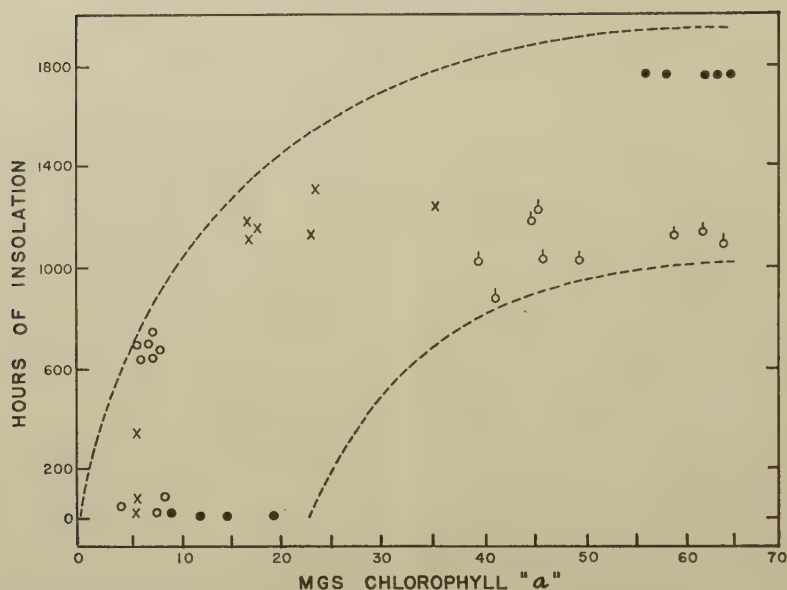


FIG. 9. Relationship between duration of insolation on artificial "rocks" and the standing crop of chlorophyll in algal growth produced during a 7-month period of immersion (August to March inclusive). The crosses represent data from Red Banks; open circles, Chokecherry; tailed circles, Canyon Road; and filled circles, Mendon Bridge. The area between the dotted lines suggests the range of chlorophyll values at a given duration of insolation on Logan River. All "rocks" receiving less than 200 hours of insolation were artificially shaded by bridges.

ning and Juday 1941). The average chlorophyll content of these lakes was 32 mg per m² of surface.

It appears that the maximum amount of chlorophyll in aquatic habitats occurs in benthic communities where the water itself or materials in suspension or solution do not seriously decrease the radiation reaching the benthos.

Chlorophyll crops on concrete "rocks" were measured to determine the effect of shading and diffusion on the standing crop produced from the end of one runoff period to the beginning of the next one. These 2 habitat factors appeared to afford the best hypotheses to explain the observed patchy and discontinuous distribution of algae on the riverbed. Chlorophyll measurements are used as the criteria for comparison in the following discussion, because all dry algal biomass estimates would be necessarily derived from them. From the standing crops of chlorophyll, productivity is also inferred; however, it must be admitted that the range of standing crop measurements might be greatly narrowed in a

situation where cropping by current attrition tended to set a limit to standing crop.

The effect of varying periods of insolation during the 7-month immersion of concrete "rocks" is suggested in Figure 9. Artificial restriction of insolation to less than one half of the maximum value possible under the existing natural conditions was obviously limiting to the chlorophyll crop. The natural variations in insolation at any station were not clearly related to distribution of chlorophyll. The results of the experiments performed above the impoundments are not comparable with those below because of differences in other variables not considered here.

Random placement of the concrete "rocks" was relied on to provide a gradient of insolation values representative of the natural variation occurring. It is evident that the natural range is not so great as one might suspect, however. More could have been learned concerning the insolation-chlorophyll relation if additional measurements between 200 and 1100 hours of insolation had been made. If a regression of

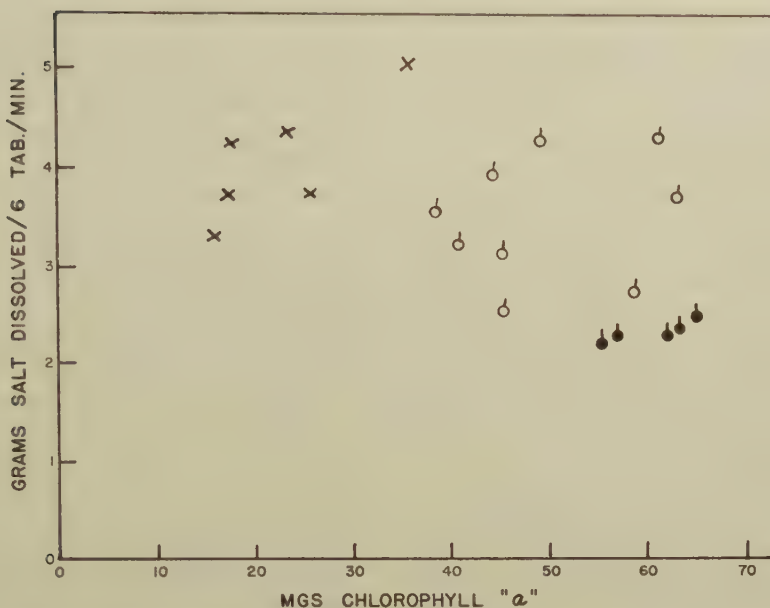


FIG. 10. Relationship between average relative diffusion rates over the surface of artificial "rocks" and standing crop of chlorophyll produced in 7 months. No values from rocks having chlorophyll crops apparently limited by shade are included in the analysis. Station codes are: crosses, Red Banks; tailed open circles, Canyon Road; tailed filled circles, Mendon Bridge.

chlorophyll on insolation does occur, this is the range where its detection appears most likely.

In the event that actual energy income was considered in Figure 9 rather than hours of insolation, the suggested parabolic relationship would become even more pronounced. In an energy based analysis 600 hours of mid-day insolation would have an energy value higher than one third of that of an 1800 hour period of insolation while complete shade measurements would still be plotted close to the base line. Analysis of chlorophyll in relation to duration of insolation received during the 3 months immediately preceding the removal of the concrete "rocks" from the stream yielded a relationship essentially the same as that in Figure 9.

The relative diffusion rates over the surfaces of the "rocks" were not convincingly related to the amounts of chlorophyll produced (Fig. 10). This was true among "rocks" at a station and between-station averages of chlorophyll standing crop. Presumably the minimum rates of diffusion present were sufficient to entirely overcome

local depletion of nutrients. Butcher (1940) observed a more abundant growth of algae on glass slides that had been placed in the more rapid sections of the stream than those from slower water and discussed this phenomenon as a generality. The largest standing crops of chlorophyll on artificial "rocks" and inferentially the highest productivities in Logan River occurred in the slowest section of the river, though chlorophyll at one of the more rapid sections almost equaled this growth. The productivity, based on oxygen production, of the third impoundment of Logan River is apparently far greater than that of an equal area of the river above it or below it. In this habitat, velocities are very low. Back eddies and dead waters behind boulders of the canyon section are observably less vegetated than rapid waters close by, but this seems to be caused by cyclical deposition and scouring of bottom materials in these locations. Small, isolated, slow-water areas in rapid streams suffer heavy deposition of the coarse materials which rapid streams are capable of carrying. It is possible that relative rather than absolute velocity

may have the more important effect on stream productivity. More permanent silted areas that occur where the level of the third impoundment causes the river to lose velocity are colonized effectively by *Ranunculus*. As pointed out by Blum (1956) true river algae do not prosper on other than stony bottoms and generally seem more abundant in rapid water, but this observation cannot be extended to mean that rapid rivers are more productive than slow rivers. It is possible that apparently heavier growths of some algae in rapid water may be a reflection of their inability to compete in a mixed community where vascular plants might be highly competitive.

Apparently more important than duration of insolation or rate of diffusion was the placement of the concrete "rocks" with respect to the impoundments on Logan River. At equal durations of insolation the lowest chlorophyll values from the stations below the impoundments exceeded the greatest values on concrete "rocks" placed at Red Banks station on the canyon section. The Chokecherry values on the canyon section are all from concrete "rocks" subject to limited insolation and are not comparable to other values with respect to position in the river. It is probable that no increase in chlorophyll on concrete "rocks" over the Red Bank values would have been noted had measurements been

made at an equally insolated location on the canyon section immediately above the impoundments, because no downstream increase in chlorophyll was noted in samples from the natural substrata within the canyon section.

Total dissolved solids and individual ions that were measured do not appear closely related to the average quantity of chlorophyll on concrete "rocks" at a station (Fig. 8). The only ions that increased downstream at about the same rate that standing crop of chlorophyll did were sulfate and chloride. There is no logical reason to expect that these ions would have a beneficial effect on algae; however, dissolved substances in trace amounts coming from the same source as sulfate and chloride may have stimulated algal growth.

Turbidity was highest and lowest at the 2 stations having the highest standing crops of chlorophyll on concrete "rocks" as well as on natural rocks (Fig. 8). The effect of turbidity is not to be confused with that of the coarser bed load carried by the canyon section above the impoundments. These molar materials are dropped out in the impoundments above Canyon Road and are not present at Mendon Bridge because of reduced velocity.

Temperatures at the 4 stations at which the artificial "rocks" were located did not afford an explanation for the apparent higher

TABLE 1. Chlorophyll-based rates of photosynthesis of algae in light and dark jar experiments

Date	Gross productivity mgO ₂ /hr./mg Chlor. <i>a</i>	Water temperature °C.	Hours of experiment	Light conditions	Dominant algae
Feb. 6	1.1	4.5	13:00-16:00	Thin overcast	Encrusting bluegreen and <i>Palmella</i>
Feb. 9	0.6	10.0	13:30-15:30	Sun	Encrusting bluegreen, <i>Vaucheria</i> and <i>Palmella</i>
Feb. 12	0.5	10.5	15:30-17:00	Sun	Encrusting bluegreen
June 29	0.7	11.5	14:00-16:00	Sun	Encrusting bluegreen
June 30	2.1	11.5	13:30-17:30	Sun	<i>Oscillatoria</i> ¹
June 30	0.7	11.5	13:30-17:30	Sun	<i>Vaucheria</i>
June 30	2.7	11.5	13:30-17:30	Sun	<i>Prasiola</i> , detached from rock
July 2	0.3	11.5	15:00-19:00	Overcast	Encrusting bluegreen
July 4	0.7	12.0	10:00-16:00	Sun	Encrusting bluegreen
July 9	1.0	12.0	14:30-16:30	Sun	<i>Chlorella</i> ²
July 22	0.07	12.5	17:00-19:30	Shade	Encrusting bluegreen
July 22	-0.14	12.5	17:00-19:30	Shade	<i>Oscillatoria</i> ¹

¹ From artesian standpipe.

² Dense culture from aquarium.

productivity of the "rocks" at the lower stations. From October to the end of the experiment in March there were no notable temperature differences among stations (Fig. 7). Anchor ice was never present at the lower 2 stations, and this may be a partial explanation for the superior standing crops of chlorophyll here.

Chlorophyll-based productivity

The theory upon which the light and dark jar experiments reported in Table 1 are based was developed for plankton communities. It is pertinent at this point to consider the basis for comparisons stated or implied in discussions of community photosynthesis.

Although the photic zone of a clear water plankton community may be many meters in thickness, it is still comparable to the thin crusts or mats of the Logan River community in which almost complete light extinction occurs. The complete community in any aquatic situation is defined here as all plant material within the photic zone. To be consistent with this convention, then, all comparisons must be made between samples representing the entire vertical series of plants in the photic zone and not between fragments "out of context." It is realized that the extent of the photic zone may be difficult to determine with precision. The term as used here applies only to the approximate zone in which enough light remains to cause significant photosynthesis.

Light saturation or actual depression of photosynthesis is known to exist in plants exposed to bright sunlight but is probably not detectable in measurements made on complete communities. Evidence indicating that light is always limiting to community photosynthesis is summarized in Figure 11. Experiments on the relation of photosynthesis of several benthic plants to light intensity conducted by Manning *et al.* (1938) would seem to indicate that benthic plants do not photosynthesize at a correspondingly greater rate when natural light intensities exceeded about 3000 foot candles. These were measurements of photosynthesis of plant fragments removed from the community, however, and are

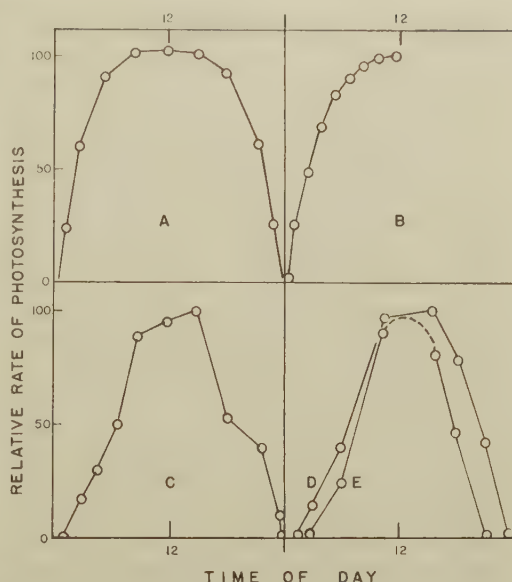


FIG. 11. Curves of photosynthetic response of various total aquatic communities to changes in solar altitude and consequent changes in incident energy. Curve A is the relative photosynthesis calculated for Atlantic plankton during 2 summer days (Ryther 1956a); curve B, Weber Lake plankton (Manning and Juday 1941); curve C Silver Springs benthos (Odum 1956); curve D, benthos of third impoundment of Logan River; and curve E, benthos of canal diverted from the first impoundment of Logan River. Asymmetry of benthic curves is probably due to unequal morning and afternoon shading.

probably not representative of the entire community of which they were part. Edmondson (1956) similarly discusses noon-time suppression of total photosynthesis of plankton communities in shallow clear lakes, but here again complete community photosynthesis is not being considered. If the photosynthesis of the benthos were added to that of the plankton it is doubtful if noontime suppression would prevail in this situation. The experiments conducted during this investigation (Table 1) on photosynthesis as related to chlorophyll content were made on complete communities as defined here with the exception of those using *Prasiola*, *Oscillatoria*, and *Chlorella*. These latter experiments on plants removed from the community of which they were part were made to aid in the analysis of the

values for the rock supported, complete communities which follows.

It is apparent that the limited number of light and dark jar experiments on which the photosynthetic value of chlorophyll is based in this investigation can only lead to an approximation. While no attempt is made to report a precise value the order of magnitude is suspect, and before applying the chlorophyll based values of photosynthesis to total production in Logan River it is desirable to consider this suspicion.

Gross primary productivity in the experiments made on representative portions of the encrusting bluegreen and *Vaucheria* communities of Logan River ranged from 0.5 to 0.7 mg O₂/hr./mg chlor. *a*. Although the lower values were correlated with a low winter sun and the higher values were made in bright summer sunlight, the narrow range despite an approximate four-fold difference in light intensity is not consistent with the apparent ability of complete communities to photosynthesize in rough proportion to all intensities of sunlight. Suspicion that photosynthesis was suppressed in the jar experiments is further aroused by the relatively high value obtained in one experiment in which agitation was provided with a battery-operated mixer (Table 1, February 6), and by the higher values prevailing in experiments on detached plant fragments and *Chlorella*. In these latter jar experiments the photosynthesizing algae were not attached to rocks, and the jars containing them were therefore agitated by the stream currents due to the resulting lower specific gravity of the jar and contents. During the experiment these plants were observed to move freely about the jar. Gessner (1937) and Odum (1957a) both report similar experiences wherein plant metabolism in confined, still water was suppressed. It is probable that removal and replacement of metabolic gases and dissolved solids by diffusion in still water cannot keep pace with photosynthesis proceeding at a high rate. This is further substantiated by unpublished data from experiments on benthic communities of Laguna Madre in Texas in which metabolisms of unconfined communities ranged from 2 to 3 times

greater than those in bell jars over representative portions of the community on the same days. From the foregoing it appears probable that an average ratio between 1 and 2 mg O₂/hr./mg chlorophyll *a* is closer to the true value than ones obtained from the jar experiments. To compute the productivity of the canyon section of Logan River a ratio of 1.5 is used.

The estimated total amount of chlorophyll *a* on the 31.7 hectares (79 acres) of the canyon section river bottom was 95 ± 19 kg at the 95 per cent confidence interval. Average annual duration of direct insolation on clear days was about 1300 hours. This estimate is based on measurements made at 6 random situations on the canyon section river bed of each of 2 typical stations. In addition to the period of direct insolation there were about 3100 hours of reduced illumination occurring during twilight, cloudy days, and periods of bright shade. Under conditions of illumination less than that of direct insolation photosynthesis probably proceeded at widely varying rates. An average rate of one-half that of sunlit periods is a reasonable estimate. This increment of photosynthesis may be incorporated into the total by adding one half of 3100 hours to the sunlit period of 1300 hours for an effective period of insolation of 2850 hours.

The estimate of gross primary production of the canyon section may now be estimated thus: $(1.5 \text{ kg O}_2/\text{hour of insolation/kg chlorophyll } a) \times (95 \text{ kg chlorophyll } a) \times (2850 \text{ hours insolation}) = 406,125 \text{ kg O}_2$ or about 118,000 kg C fixed at a PQ of 1.25.

This production, when converted to a rate, may be compared to productivity of 3 other stations on Logan River and 3 other published values in Table 2. The estimate for the third impoundment is not corrected for diffusion loss. Although low in gross primary productivity when compared to other moving water habitats, the value for the canyon section exceeds that of a moderately productive lake. The high figure for the third impoundment of Logan River is in accord with the good reputation it has for production of brown trout. Rooted

TABLE 2. Comparative rates of gross primary production

Glucose produced kg/m ² /year	Water	Reference	Remarks
1.2	Canyon section of Logan River	—	Average rate based on average of fall and late winter chlorophyll/m ²
4.6	Third impoundment Logan River	—	Average rate by diurnal oxygen curve, following method outlined by Odum (1956)
5.0	Canyon Road (below first impoundment Logan River)	—	Average rate based on chlorophyll/m ² as determined from 2 fall and 2 spring transects
3.1	Mendon Bridge, last riffle before valley base level	—	Average rate based on chlorophyll/m ² as determined from 1 spring transect
8.8	Eniwetok Atoll	(Odum & Odum 1955)	Diurnal oxygen curve determination
0.6	Weber Lake	(Manning & Juday 1941)	Maximum summer photosynthesis of most productive of several Wisconsin lakes
0.2-21.5	11 Florida Springs	(Odum 1957b)	Diurnal oxygen curve determinations

TABLE 3. Chlorophyll *a* content of several algae from Logan River and vicinity

Algae	Remarks	Per cent <i>a</i> chlorophyll based on dry weight
<i>Cladophora</i>	February	0.55
<i>glomerata</i>	March	0.69
<i>Palmella myo-</i>	February—vigorous	0.98
<i>surus</i>	March—senescent	0.15
<i>Vaucheria</i>	February	0.70
	March	1.05
<i>Lyngbya</i> & <i>Chlorotylum</i>	From aquarium	2.40
<i>Oscillatoria</i>	From artesian stand- pipe, June	1.50
<i>Oscillatoria</i>	From artesian stand- pipe, July	1.30

vascular aquatics and their Aufwuchs are the preponderant producers in this sluggish river type of environment.

Net productivity and standing crop

The ratio of net primary production to gross primary production in natural communities is not determinable with present field techniques. It is conceivable that a community of plants might use the entire product of photosynthesis in respiration or only 10 per cent of it as found for vigorous cultures of plankton algae (Ryther 1954). Verduin (1956) states that the autotrophic community of Lake Erie respired 80 per cent of gross primary production. With

no other evidence on hand a reasonable first approximation of net production in Logan River can be gained by assuming a respiration value midway between the foregoing extremes, or 45 per cent. Net production may then be computed as 77,000 kg of C or 154,000 kg of organic matter if *C* is considered to comprise about 50 per cent of most organic matter (Ryther 1956b).

The comparison between average standing crop of algae and annual net production may be made by using the chlorophyll-dry weight ratio to determine standing crop. The use of chlorophyll content to estimate plant biomass is subject to errors caused by variation in the chlorophyll-dry weight ratio. Rabinowitch's tabulation (1945) of proportions of chlorophyll *a* determined by several investigators to be in green and bluegreen algae indicates a range of 0.09 to 2.00 per cent of the dry weight. If laboratory cultures (*Chlorella*) are excluded the range is reduced. During the course of the present investigations the range of the proportion of chlorophyll *a* in some algae of Logan River and vicinity was from 0.15 per cent to 2.4 per cent (Table 3).

Because the species composition and condition of senescence of algal communities were observable in communities sampled from Logan River, it was possible to use the applicable chlorophyll-dry weight ratio correlated with species composition and

condition, or an approximate weighted average of applicable values, in estimating the dry plant biomass. In the canyon section of Logan River, a weighted average (1.15 per cent) of the values for *Vaucheria*, *Oscillatoria* (a substitute for encrusted blue-green algae), and young *Palmella Myosurus* was used.

The estimated, average, dry plant biomass of the canyon section was 8,000 kg or 25 grams per m². No correction was made for ash in the dry weight of algae used to determine the chlorophyll-dry weight relationship. If an arbitrary correction of 10 per cent ash is used, the average standing crop is equal to 4.7 per cent of the ash-free dry weight of yearly net production.

An alternate analysis of the disposition of gross primary production is available if the estimate of total community respiration from the dark jar experiments is used. By considering respiration to have been repressed to the same degree as photosynthesis during the light and dark jar experiments, an annual figure for combined heterotrophic and autotrophic respiration of 376,000 kg O₂ may be computed for the canyon section. When this quantity is subtracted from the estimate of the O₂ equivalent of gross primary production, a remainder of about 28,000 kg is left. With a PQ of 1.25 the O₂ remainder is equal to 8,200 kg of C or about 16,000 kg of organic matter to be accounted for in biomass increase and export. The yearly change in biomass is probably small relative to the order of magnitude of other categories being considered. The dry weight of the total biomass of the canyon section may be roughly estimated at 10,000 kg, and in an unchanging habitat like Logan River it probably varies little when measured at the same time of year. A crude estimate of export at the end of the canyon section of 28,000 kg was made using the dry plant weight equivalent of chlorophyll in stream drift. Theoretically this figure is minimal since it excludes animal material and dissolved organic matter, and underestimates the biomass of senescent and decomposing algal fragments. It may be concluded then that the ratio of respiration to photosynthesis is in error to some degree because the

difference between them will not account for export. Further extension of this analysis is meaningless because of the cursory nature of many of the measurements made and the number of assumptions involved.

CONCLUSIONS

In the absence of other parameters it appears that chlorophyll content may be used as an indicator of the order of magnitude of productivity of lithophilic algae in rapid streams. Knowledge of the total-chlorophyll in a habitat also allows the estimation of plant biomass. These data taken at appropriate intervals plus an adequate measurement of export and import of organic matter and community respiration should allow the estimation of the gain in animal biomass (including any removal or harvest) during a chosen period. Direct measurements of change in biomass of portions of the total animal biomass would allow estimation of any fraction not easily measured. Although only an approximation, the gain of fish biomass estimated in this fashion would be enlightening to fishery managers concerned with stream production. A series of such measurements from a variety of streams might indicate the relative efficiencies of differing food webs as fish producers.

Chlorophyll sampling on a simple, random basis seems justified in streams in view of the lack of any clear relation between chlorophyll standing crop and intimate water currents or degree of shading within the normally occurring range of values.

The greatest utility of the method would be in assessing productivity of algae growing as mats, films, or crusts. Where the streamer or foliose growth habit is common or where vascular plants are abundant, chlorophyll extraction becomes a problem. It is probable that the latter forms would occur in streams where the diurnal oxygen curve method would be applicable.

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NOTES AND COMMENT

A Planktonic Fish Egg from Fresh Water¹

INTRODUCTION

Statements that pelagic fish eggs occur only in species of marine fishes are common in the literature (e.g., Bridge 1932, Brown 1957, Davis 1955). Apparently, freshwater pelagic eggs previously have not been reported from temperate regions. On the other hand, Hora and Pillay (1955) listed pelagic eggs for three species of freshwater fish that are raised commercially in ponds in the Indo-Pacific region: *Helostoma temminckii* (kissing gourami), *Anabas testudineus* (climbing perch), and *Mugil corsula* (grey mullet). The last mentioned occurs in estuarine and coastal waters, as well as in fresh water. In addition, Mookerjee (1945) has listed floating eggs for certain Indian freshwater species (*Ophicephalus punctatus*, *O. striatus*, *O. marulius*, *Anabas testudineus*, *Colisa fasciata*, and *C. lalius*). It is not clear that these are all planktonic (Hora and Pillay list the species of *Ophicephalus* as nest builders). Daiber (1950), whose work has been summarized by Langlois (1954), suspected that the eggs of the sheepshead (*Aplodinotus grunniens*) in Lake Erie were pelagic. He reported, on the basis of oral information from the director of the Toledo (Ohio) Aquarium, that viable eggs were produced in the sheepshead tank in the aquarium. These eggs floated to the top, and most of them were lost in the overflow. Daiber gathered anatomical evidence that the spawning season of the sheepshead was long. The major spawning of females took place after July 1st, but he found a recently spent female as late as September 11th. In his investigations he was unable to determine spawning grounds, but young-of-the-year were collected in Sandusky Bay (mostly in shallow, weedy areas at the upper end of the bay). Many other young-of-the-year were collected, however, in deep open water in the vicinity of the Bass Islands, and he thought it likely that the fish spawned in the open lake areas, as well as in Sandusky Bay.

There is no *a priori* reason that freshwater pelagic eggs should not occur in temperate as well as tropical regions, although because of the constant flow of fresh waters towards the sea, fishes having such eggs would find them somewhat disadvantageous. Therefore relatively few species can be expected to have this means of dissemination of their sex products.

COLLECTIONS

On July 3, 1958, a single pelagic fish egg was picked from a routine plankton sample collected in Hatchery Bay (Put-in-Bay), in western Lake Erie. The egg floated at the very surface of the water. This egg hatched between 15½ and 16½ hours after collection.

Between July 4th and July 8th, 5 additional viable eggs, and 4 that were disintegrating (unfertilized?) were captured by dragging a plankton net at the surface with the upper half of the net out of the water. These eggs were all collected in Hatchery Bay and Squaw Harbor on days when the wind was blowing onshore. Attempts to capture eggs when the wind was offshore were unsuccessful.

On the evening of July 7th a living female sheepshead from Peach Point (at the entrance to Hatchery Bay) was brought into Dr. Howard Clemens' laboratory at the Stone Laboratory. The fish spawned extensively in a laboratory tank. Her eggs were not fertilized, and hence soon degenerated, but they were the same as the viable pelagic eggs that had been captured in the plankton in size and in their proclivity to float at the very surface of the water, and they were identical to the disintegrating planktonic eggs. The author wishes to acknowledge with sincere thanks Dr. Clemens' cooperation in making these eggs available.

All of the viable eggs that were allowed to develop hatched less than 24 hours after capture. Hatching occurred in the early morning hours or during the night, and the exact time of hatching was observed in only one instance. This one egg, however, was at such an early stage of development at the time of capture that no trace of the primitive streak could be observed from the ventral view (due to the large ventral oil droplet, all of the eggs floated ventral side up, but the oil and yolk were so transparent that the embryos could be seen through them at a fairly early stage). This one egg hatched 22 hours after capture. Judging by the early stage of its development when collected, the total time of embryonic life lay perhaps between 25 and 30 hours after spawning and fertilization (the temperature was 22°C.). This is a very short developmental period for temperate freshwater fish eggs, though comparable to that of some of the tropical eggs, both pelagic and otherwise, reported by Hora and Pillay (1955).

The ephemeral membership in the meroplankton probably accounts in part for the failure to discover the eggs of the sheepshead in the plankton heretofore even though the fish is very common in Lake Erie. This failure, however, remains somewhat surprising in view of the facts that: 1) the plankton has been examined by generations of students and instructors at the F. T. Stone Lab-

¹ This study was incidental to an investigation of planktonic Lake Erie copepods, which was supported by a grant from the Ohio Division of Wildlife, administered by the Natural Resources Institute of Ohio State University. Thanks are extended to Dr. D. G. Frey for information concerning, and for the loan of, literature that otherwise was unavailable.

oratory at Put-in-Bay during the spawning season of *Aplodinotus*, 2) there have been intensive special studies of the plankton in the area (Chandler 1940, Wright *et al.* 1955), and 3) an intensive search was made in the plankton for precisely these eggs (Daiber 1950).

GENERAL DESCRIPTION OF THE EGG AND EARLY FRY

Detailed descriptions of the embryonic development and fry of *Aplodinotus grunniens* must await the availability of adequate quantities of material, whose exact age after fertilization is known. However, it is felt that general descriptions of the egg and fry are desirable here.

As is characteristic of pelagic fish eggs, such as are to be found in the marine plankton (*e.g.*, see Ehrenbaum 1905-1909), the eggs under consideration were small compared to the size of demersal (and other) eggs, the fluid-filled spaces between the yolk mass and the outer egg membrane (chorion) were very extensive, and each had a large oil droplet, both to serve for the storage of nutrient material and to increase the buoyance of the egg.

In the eggs that were collected, the diameters of the outer membranes varied from 1.15 to 1.70 mm, the yolks varied in diameter from 0.92 to 1.10 mm, and the main oil droplets were from 0.64 to 0.72 mm (in two of the viable eggs there were additional smaller oil droplets, as shown in Figure 1). Usually the yolk mass was placed (as seen

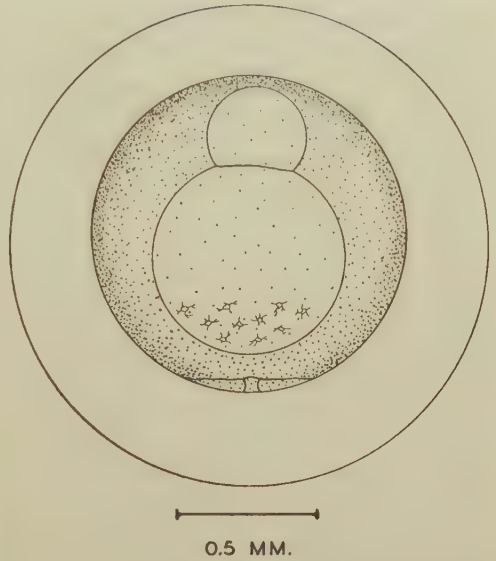


FIG. 2. Another egg, approximately three hours later in development than that shown in Figure 1. The beginning of the development of the head can be observed. The oil drop is double, perhaps having been divided by rough treatment during capture. Later the two droplets fused into one. Chromatophores are beginning to develop dorsal to the oil drop.

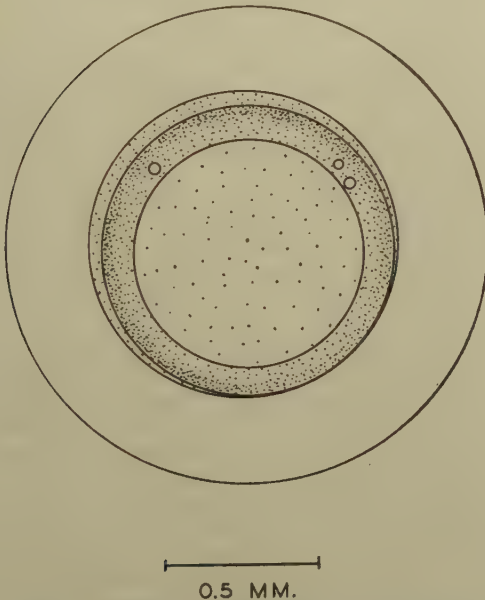


FIG. 1. Egg in earliest stage of development encountered. In addition to the single large oil drop, there are three tiny ones. This egg hatched 22 hours after the drawing was made.



FIG. 3. Egg approaching the hatching stage. The number of myomeres in the tail region could not be determined accurately in the living specimen. Note the development of scattered chromatophores dorsal to the oil drop and on the surface of the yolk mass.

from a ventral view) in the very center of the fluid-filled chorion, but in one instance it was eccentric. The oil droplet likewise was usually centrally located with respect to the yolk mass, but in one instance it also was eccentric. (There were two oil droplets in this specimen, as shown in Figure 2, and the same egg was the one with the eccentrically-located yolk mass, leading one to suspect the egg had received rough treatment during capture in the plankton net. In this egg the two oil drops later coalesced to form one.)

In the egg at the earliest stage of development observed (Figure 1) no sign of the primitive streak could be detected from the ventral view, and no pigmentation was apparent anywhere in the visible portion of the egg. In about three hours the developing head region became clearly visible, comparable to that shown in another egg in Figure 2. Still there was no sign of pigmentation, but a few colorless cells, evidently destined to become chromatophores, had become visible dorsal to the oil drop and on the yolk mass lateral to the oil drop. Later, both head and tail of the embryo were clearly visible, and about this time there was a group of scattered chromatophores containing dark pigment dorsal to the anterior half of the oil drop. Similar cells, which however did not contain pigment, could be seen through the oil drop near the equatorial region, but the posterior half was devoid of such cells.

Still later the tail region became loosened from the yolk mass (see Fig. 3). The chromatophores dorsal to the oil drop continued to spread, and eventually the yolk that could be seen through the drop was covered (rather sparsely) with them. They were particularly evident because the oil drop acted as a magnifier that brought them sharply and clearly into focus. A few chromatophores, unmagnified by the oil drop, lay on the ventral (upper) surface of the oil drop, especially towards the end of embryonic development, and there was a group of them on the yolk mass at each side of the head.

Movements of the tail and body commenced a couple of hours before hatching, and at about the same time the heart could be seen contracting rhythmically. The blood, however, was not pigmented. At the time of hatching there was a small red-pigmented region laterally between the eye and the ear capsule on each side. There was no other pigmentation except the sparse scattering of chromatophores mentioned above.

As hatching time approached, more and more of the tail became freed from the yolk mass. This was accompanied by a shifting of the orientation, such that the head rotated down and the tail rotated up. The oil droplet shifted towards the posterior portion of the yolk mass. Hatching itself (observed only once) occurred tail first.

The newly-hatched fry were around 4.2 mm in length. They were very active, swimming by vigorous lashing of the tail. Pelvic fins were not

evident. The dorsal fin extended the length of the body, and was continuous with the caudal fin, which in turn continued ventrally to the anal region. No fin rays were to be seen in any of these fins. The straightening of the body of the embryo forced the yolk mass into an oval shape. The oil drop, which remained approximately the same size as before, continued the movement towards the posterior part of the yolk mass that was initiated before hatching, until it came to lie just anterior to the anus. The fry still maintained their embryonic position, ventral side up, except that the shifted position of the buoyant oil drop forced them to hang at a 45° angle, with the head down. As in the eggs, the specific gravity of the fry was considerably less than that of water, and hence they floated towards the surface whenever they ceased their swimming activities.

Pelagic fish fry were captured a number of times in plankton samples, but invariably they were so badly macerated in the crowded conditions of the sample that it is unsafe to attempt to compare them, either with the fry emerging from the pelagic eggs, or with the sheepshead fry described by Daiber (1950) and Fish (1932). Daiber's smallest fry, tentatively identified as sheepshead, was 7.85 mm long, whereas the smallest obtained by Fish was 13.3 mm.

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BOOK REVIEWS

HOUGH, JACK L. 1958. *Geology of the Great Lakes*. Univ. Illinois Press, Urbana. xviii + 313 pp., 75 figs., 22 tables. \$8.50.

A devotee of the Great Lakes since his boyhood, it is entirely fitting that Jack Hough should produce the first full treatment of Great Lakes glacial history since the monographic work of Leverett and Taylor in 1915 (*The Pleistocene of Indiana and Michigan and the History of the Great Lakes*. U. S. Geol. Surv. Monograph 53). Hough writes in a lucid simple-English prose, and the publishers have complemented his style by providing a clean-cut simple cover and binding. I found the whole to be very well done.

The book is presented in essentially two parts. Approximately the first half is a straightforward nontechnical review of the modern Great Lakes, the preglacial history of the region, Pleistocene glaciation, and the several means by which events in the Lakes' histories are dated. The second half deals with the details of the histories of the lake-stages. This section is technical in its approach and in the detail and rigor of its reasoning, but is well written and the attentive non-geological reader should have little difficulty in following it. The author here deals with a wealth of detail, but by subdivision into sections dealing with each lake separately he inconspicuously obtains the reiteration necessary for the orientation and instruction of the reader. His cross-referring to conditions in other lakes during the discussion of

each lake facilitates the reader's use of the 26 lake-stage maps wherein conditions in the several lakes are integrated. These maps contain a great deal of information and will be widely used.

There can be little doubt that the author has been successful in his avowed (p. viii) purpose of revising and up-dating the glacial history of the Great Lakes. His pointing out of areas of data-lack, areas of conjectural interpretation, and areas of disagreement should do much toward the stimulation of research in Great Lakes glacial geology.

Aside from its primary geological function, this book will be both informative and useful to limnologists and fishery biologists. The various and varying outlets of the Great Lakes stages have long been known to have provided means for the distribution of aquatic species. This most recent summary of drainages and drainage-connections may contribute new light to the distributions of water organisms. It also appears that the time is about right for an examination of species-distribution data to see if they have any collateral uses in clarifying dubious points in the geological history.

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American Society of Limnology and Oceanography

Special Publication No. 1

Sources of Limnological and Oceanographic Apparatus and Supplies

Revised and enlarged, April, 1959 by Committee on Apparatus and Supplies

J. H. Ryther, Chairman

C. S. Yentsch

G. H. Lauff

This edition of Special Publication No. 1, as before, consists of two lists. In the first of these there are presented alphabetically the various types of apparatus and supplies. Following each item are numbers which refer to the firms that manufacture or distribute that item. The names of these firms, together with the numbers that identify them, are arranged in alphabetical and numerical order in the second list.

In preparing this revision, the Committee has eliminated most items not used specifically and uniquely in Limnology and Oceanography. For example, meteorological instruments are omitted from this edition as are most items which may be found in general scientific supply house catalogs or classified directories such as Thomas' Register of American Manufacturers.

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89 Commerce Road
Cedar Grove, New Jersey
66. Ingersoll-Rand Co.
11 Broadway
New York 4, New York
67. The C. O. Jelliff Mfg. Corp.
Southport,
Connecticut
68. Kaar Engineering Corp.
2995 Middlefield Road
Palo Alto, California
69. Kelvin & Hughes (Marine) Ltd.
90 Fenchurch Street
London E. C. 3,
England
70. Walter H. Kessler Co.
219 Westbury Ave.
Carle Place
Long Island, New York
and,
Kessler, Inc.
3801 Barnett Street
Houston 17, Texas
71. Ketchum Mfg. Co., Ltd.
P. O. Box 388
Westboro, Ottawa 3,
Ontario, Canada
72. Kolstrand Mfg. Co.
4739 Ballard Ave.
Seattle 7, Washington
73. Kressilk Products, Inc.
73 Murray Street
New York 7, New York
74. Laboratoire Oceanographique
P. O. Box 19
Charlottenlund Slot
Charlottenlund, Denmark
75. La Motte Chemical Products Co.
Chestertown, Maryland
76. Ledeen Mfg. Company
Gilman Road at Garvey Blvd.
El Monte, California
77. Leeds & Northrop Co.
4970 Stenton Avenue
Philadelphia 44, Pennsylvania
78. E. Leitz, Inc.
468 4th Avenue
New York 16, New York
79. Leupold and Stevens Instruments Inc.
4445 N. E. Glisan Street
Portland 13, Oregon
80. The Linen Thread Co., Inc.
418 Grand St.
Paterson 1, New Jersey
81. M. & E. Marine Supply Company
P. O. Box 601
Camden 1, New Jersey
82. Macalaster-Bicknell, Inc.
243 Broadway
Cambridge, Massachusetts
83. Marine Instruments Ltd.
107 Fenchurch St.
London Ec 3, England
84. Marine Machine Works
2021 Avenue B
Galveston, Texas
85. Markey Machinery Co., Inc.
85 Horton Street
Seattle 4, Washington
86. Membranfiltergesellschaft
Weender Landstrasse 96/102
Göttingen, Germany
87. Meteor Instrument Co.
P. O. Box 121
Annapolis, Maryland

88. Miller-Dunn Co., Inc.
2517 N. W. 21st Terrace
Miami, Florida
89. Millipore Filter Corp.
Bedford,
Massachusetts
90. Minneapolis-Honeywell Regulator Co.
Seattle Development Lab.
1121 Westlake
Seattle 9, Washington
91. Mission Instruments Co.
2836 Deerpark Drive
San Diego 10, California
92. Morse Diving Equipment Co., Inc.
51 Sleeper Street
Boston 10, Massachusetts
93. National Band and Tag Co.
721 York Street
Newport, Kentucky
94. National Institute of Oceanography
Wormley near Godalming
Surrey, England
95. National Steel and Shipbuilding Corp.
Harbor Drive at 28th
San Diego 12, California
96. Negretti and Zambra, Ltd.
122 Regent Street
London W 1, England
97. Newark Wire Cloth Co.
351 Verona Ave.
Newark 4, New Jersey
98. New England Trawler Equipment Co.
305 Eastern Ave.
Chelsea 50, Massachusetts
99. Nurnberg Thermometer Co.
124 Livingston St.
Brooklyn, New York
100. Olympic Instrument Laboratories
Vashon, Washington
101. A. Ott
Kempton, Bayern,
Germany
102. S. B. Penick and Co.
50 Church Street
New York 8, New York
103. The Plastic Film Co.
116-118 Market Ave. E.
Winnipeg 2, Manitoba
104. Precision Thermometer and Instru-
ment Co.
1434 Brandywine Street
Philadelphia 30, Pennsylvania
105. W. R. Prior and Co., Ltd.
28a Devonshire Street
London W-1, England
106. Prodelin, Inc.
307 Bergen Avenue
Kearny, New York
107. Puget Sound Workshop
3311-110th S. E.
Bellevue, Washington
108. Pye Telecommunications Ltd.
Newmarket Road
Cambridge, England
109. Radio Corporation of America
Engineering Products Division
Camden, New Jersey
110. Ray's Machine Shop
360 Wesley Street
Nanaimo, B. C., Canada
111. Raytheon Mfg. Co.
Submarine Signal Division
River Street
Waltham, Massachusetts
112. C. Reichert
Optische Werke
Wien XVII
Hern. Hauptstr. 219
Vienna, Austria
113. Richter and Wiese
Kreuzbergstrasse
Berlin SW 61, Germany
114. Rigosha Mfg. Co., Ltd.
2 Kajicho 1-chome,
Kanda, Chiyoda-Ku,
Tokyo, Japan
115. Salt Lake Stamp Co.
43 West Broadway
Salt Lake City, Utah
116. Carl Schleicher and Schuell Co.
Keene, New Hampshire
117. William C. Schroeder
Woods Hole,
Massachusetts
118. E. Fred Schueler
30 Albermarle Road
Waltham 54, Massachusetts
119. Scott Aviation Corp.
Safety Equipment Division
207 Erie Street
Lancaster, New York
120. Scottish Marine Biological Association
Oceanographic Laboratory
78 Craighall Road
Edinburgh 6, Scotland

121. Seahawk Products
P. O. Box 1157
Coral Gables, Florida
122. J. H. Shepard Son & Co.
1820 East Avenue
Elyria, Ohio
123. Sprague and Henwood, Inc.
221 West Olive Street
Scranton, Pennsylvania
124. A. M. Starr Net Co.
Summit Street
East Hampton, Connecticut
125. Stevens Linen Associates, Inc.
Mill Street
Dudley, Massachusetts
126. Stroudsburg Engine Works Inc.
62 North Third Street
Stroudsburg, Pennsylvania
127. Superior-Lidgewood-Mundy Corp.
5-7 Dey Street
New York, New York
128. Tacoma Boat Building Co., Inc.
132 Siteum Waterway
Tacoma 2, Washington
129. W. A. Taylor and Co.
7300 York Road
Baltimore 4, Maryland
130. A. H. Thomas Co.
West Washington Square
Philadelphia 5, Pennsylvania
131. Tobler, Ernst & Traber, Inc.
71 Murray Street
New York 7, New York
132. Tracerlab, Inc.
1601 Trapelo Road
Waltham 54, Massachusetts
133. Tsurumi Seiki Kosakusho Co., Ltd.
1506 Tsurumi, Tsurumi-ku
Yokohama, Japan
134. W. S. Tyler Co.
3615 Superior Ave.
Cleveland 14, Ohio
135. Underwater Sports
2219 Biscayne Blvd.
Miami, Florida
136. Underwater Surveys
2928 Balboa Avenue
San Diego 9, California
137. University of Washington
Department of Oceanography
Seattle 5, Washington
138. University of Wisconsin
Department of Botany
Madison, Wisconsin
139. U. S. Divers Co.
11201 W. Pico Blvd.
Los Angeles 64, California
140. U. S. Rubber Co.
Container Products Division
Valley Street
Providence, Rhode Island
141. Wallace and Tiernan Products, Inc.
25 Main Street
Belleville 9, New Jersey
142. Ward's Natural Science Establish-
ment, Inc.
P. O. Box 24
Rochester 9, New York
143. Watanabe Keiki Mfg. Co., Ltd.
114 Higashikata-machi,
Bunkyo-ku,
Tokyo, Japan
144. Weksler Thermometer Corp.
195 E. Merrick Road
Freeport, Long Island, New York
145. Western Gear Corp.
417 9th Avenue, South
Seattle 4, Washington
146. C. H. Wheeler Mfg. Co.
Sedgley Avenue at 19th & Lehigh
Philadelphia 32, Pennsylvania
147. Wilfred O. White & Sons, Inc.
178 Atlantic Ave.
Boston, Massachusetts
148. Whitehead & Hoag Co.
277 Sussex Avenue
Newark 1, New Jersey
149. L. V. Whitney and Son
1987 Corralitos Drive
San Luis Obispo, California
150. Wildlife Supply Co.
410 Van Buren Street
Saginaw, Michigan
151. Wilkins-Anderson Co.
4525 W. Division St.
Chicago 51, Illinois
152. Wilks Precision Instruments Co., Inc.
4821 Bethesda Avenue
Washington 14, D. C.
153. The Woods Hole Oceanographic In-
stitution
Woods Hole,
Massachusetts

154. Yoshino Keiki Co.
1-14 Nishigahara, Kita-ku
Tokyo, Japan

155. Carl Zeiss, Inc.
485 Fifth Avenue
New York, New York

156. Firma Zwickert
Universitätsmechaniker
Kiel, Germany

157. Also see Fishery Leaflet 195
U. S. Department of the Interior

158. See any general laboratory supply
house catalog.

